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(71) Applicants: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US). THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US).

(72) Inventors: AUSUBEL, Frederick, M.; 271 Lake Avenue, Newton, MA 02161 (US). STASKAWICZ, Brian, J.; 18945 Marciel Court, Castro Valley, CA 94546 (US). BENT, Andrew, F.; 111 York Drive, Piedmont, CA 94611 (US). DAHLBECK, Douglas; 18851 Lenross Court, Castro Valley, CA 94546 (US). KATAGIRI, Fumiaki; Seven Hardan Road, Somerville, MA 02144 (US). KUNKEL, Barbara, N.; 6042 Pershing Avenue, St. Louis, MI 63112 (US). MINDRINOS, Michael, N.; Apartment 2, 49 Laurel Street, Somerville, MA 02143 (US). YU, Guo-Liang; 13524 Straw Bale Lane, Darnestown, MD 20878 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

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(54) Title: *RPS2* GENE AND USES THEREOF

(57) Abstract

Disclosed is substantially pure DNA encoding an *Arabidopsis thaliana* Rps2 polypeptide; substantially pure Rps2 polypeptide; and methods of using such DNA to express the Rps2 polypeptide in plant cells and whole plants to provide, in transgenic plants, disease resistance to pathogens.

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RPS2 GENE AND USES THEREOF

Background of the Invention

The invention relates to recombinant plant nucleic acids and polypeptides and uses thereof to confer disease resistance to pathogens in transgenic plants.

Plants employ a variety of defensive strategies to combat pathogens. One defense response, the so-called hypersensitive response (HR), involves rapid localized necrosis of infected tissue. In several host-pathogen interactions, genetic analysis has revealed a gene-for-gene correspondence between a particular avirulence (*avr*) gene in an avirulent pathogen that elicits an HR in a host possessing a particular resistance gene.

Summary of the Invention

In general, the invention features substantially pure DNA (for example, genomic DNA, cDNA or synthetic DNA) encoding an Rps polypeptide as defined below. In related aspects, the invention also features a vector, a cell (e.g., a plant cell), and a transgenic plant or seed thereof which includes such a substantially pure DNA encoding an Rps polypeptide.

In preferred embodiments, an *RPS* gene [SEQ. ID NO:5] is the *RPS2* gene of a plant of the genus *Arabidopsis*. In various preferred embodiments, the cell is a transformed plant cell derived from a cell of a transgenic plant. In related aspects, the invention features a transgenic plant containing a transgene which encodes an Rps polypeptide that is expressed in plant tissue susceptible to infection by pathogens expressing the *avrRpt2* avirulence gene [SEQ. ID. NO:105] or pathogens expressing an avirulence signal similarly recognized by an Rps polypeptide.

In a second aspect, the invention features a substantially pure DNA which includes a promoter capable of expressing the *RPS2* gene [SEQ. ID. NO:1] in plant tissue susceptible to infection by bacterial pathogens expressing the *avrRpt2* avirulence gene [SEQ. ID NO:105].

In preferred embodiments, the promoter is the promoter native to an *RPS* gene. Additionally, transcriptional and translational regulatory regions are preferably native to an *RPS* gene.

The transgenic plants of the invention are preferably plants which are susceptible to infection by a pathogen expressing an avirulence gene, preferably the *avrRpt2* avirulence gene [SEQ ID. NO:105]. In preferred embodiments the transgenic plant is from the group of plants consisting of but not limited to *Arabidopsis*, tomato, soybean, bean, maize, wheat and rice.

In another aspect, the invention features a method of providing resistance in a plant to a pathogen which involves: (a) producing a transgenic plant cell having a transgene encoding an Rps2 polypeptide wherein the transgene is integrated into the genome of the transgenic plant and is positioned for expression in the plant cell; and (b) growing a transgenic plant from the transgenic plant cell wherein the *RPS2* transgene is expressed in the transgenic plant.

In another aspect, the invention features a method of detecting a resistance gene in a plant cell involving: (a) contacting the *RPS2* gene [SEQ ID NO:1] or a portion thereof greater than 18 nucleic acids in length with a preparation of genomic DNA from said plant cell under hybridization conditions providing detection of DNA sequences having about 50% or greater sequence identity to the DNA sequence of Fig. 2 encoding the Rps2 polypeptide [SEQ. ID NOS:2-5].

In another aspect, the invention features a method of producing an Rps2 polypeptide which involves: (a) providing a cell transformed with DNA encoding an Rps2 polypeptide positioned for expression in the cell; (b) culturing the transformed cell under conditions for expressing the DNA; and (c) isolating the Rps2 polypeptide.

In another aspect, the invention features substantially pure Rps2 polypeptide. Preferably, the polypeptide includes a greater than 50 amino acid sequence substantially identical to a greater than 50 amino acid sequence shown in Fig. 2, open reading frame "a". Most preferably, the polypeptide is the *Arabidopsis thaliana* Rps2 polypeptide [SEQ. ID NOS:2-5].

In another aspect, the invention features a method of providing resistance in a transgenic plant to infection by pathogens which do not carry the *avrRpt2* avirulence gene wherein the method includes: (a) producing a transgenic plant cell having transgenes encoding an Rps2 polypeptide as well as a transgene encoding the *avrRpt2* gene product [SEQ ID. NO:106] wherein the transgenes are integrated into the genome of the transgenic plant; are positioned for expression in the plant cell; and the *avrRpt2* transgene and, if desired, the *RPS2* gene, are under the control of regulatory sequences suitable for controlled expression of the gene(s); and (b) growing a transgenic plant from the transgenic plant cell wherein the *RPS2* and *avrRpt2* transgenes are expressed in the transgenic plant.

In another aspect, the invention features a method of providing resistance in a transgenic plant to infection by pathogens in the absence of avirulence gene expression in the pathogen wherein the method involves: (a) producing a transgenic plant cell having integrated in the genome a transgene containing the *RPS2* gene under

the control of a promoter providing constitutive expression of the *RPS2* gene; and (b) growing a transgenic plant from the transgenic plant cell wherein the *RPS2* transgene is expressed constitutively in the transgenic plant.

In another aspect, the invention features a method of providing controllable resistance in a transgenic plant to infection by pathogens in the absence of avirulence gene expression in the pathogen wherein the method involves: (a) producing a transgenic plant cell having integrated in the genome a transgene containing the *RPS2* gene under the control of a promoter providing controllable expression of the *RPS2* gene; and (b) growing a transgenic plant from the transgenic plant cell wherein the *RPS2* transgene is controllably expressed in the transgenic plant. In preferred embodiments, the *RPS2* gene is expressed using a tissue-specific or cell type-specific promoter, or by a promoter that is activated by the introduction of an external signal or agent, such as a chemical signal or agent.

By "disease resistance gene" is meant a gene encoding a polypeptide capable of triggering the plant defense response in a plant cell or plant tissue. An *RPS* gene is a disease resistance gene having about 50% or greater sequence identity to the *RPS2* sequence [SEQ ID. NO:1] of Fig. 2 or a portion thereof. The gene, *RPS2*, is a disease resistance gene encoding the Rps2 disease resistance polypeptide [SEQ. ID NOS:2-5] from *Arabidopsis thaliana*.

By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably

95% homology to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, substitutions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By a "substantially pure polypeptide" is meant an Rps2 polypeptide which has been separated from components which naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, Rps2 polypeptide. A substantially pure Rps2 polypeptide may be obtained, for example, by extraction from a natural source (e.g., a plant cell); by expression of a recombinant nucleic acid encoding an Rps2 polypeptide; or by chemically synthesizing the protein.

Purity can be measured by any appropriate method, e.g., those described in column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes.

By "substantially pure DNA" is meant DNA that is free of the genes which, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) an Rps2 polypeptide.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of, e.g., an

Rps2 polypeptide, a recombinant protein or a RNA molecule).

By "reporter gene" is meant a gene whose expression may be assayed; such genes include, without limitation, β-glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), and ß-galactosidase.

By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific or inducible by external signals or agents; such elements may be located in the 5′ or 3′ regions of the native gene.

By "operably linked" is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

By "plant cell" is meant any self-propagating cell bounded by a semi-permeable membrane and containing a plastid. Such a cell also requires a cell wall if further propagation is desired. Plant cell, as used herein includes, without limitation, algae, cyanobacteria, seeds suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

By "transgene" is meant any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism.

By "transgenic" is meant any cell which includes a DNA sequence which is inserted by artifice into a cell and becomes part of the genome of the organism which develops from that cell. As used herein, the transgenic organisms are generally transgenic plants and the DNA (transgene) is inserted by artifice into the nuclear or plastidic genome.

By "pathogen" is meant an organism whose infection into the cells of viable plant tissue elicits a disease response in the plant tissue.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings will first be described.

Drawings

Figs. 1A - 1F are a schematic summary of the physical and RFLP analysis that led to the cloning of the *RPS2* locus.

Fig. 1A is a diagram showing the alignment of the genetic and the RFLP maps of the relevant portion of *Arabidopsis thaliana* chromosome IV adapted from the map published by Lister and Dean (1993) Plant J. 4:745-750. The RFLP marker L11F11 represents the left arm of the YUP11F11 YAC clone.

Fig. 1B is a diagram showing the alignment of relevant YACs around the *RPS2* locus. YAC constructs designated YUP16G5, YUP18G9 and YUP11F11 were provided by J. Ecker, University of Pennsylvania. YAC constructs designated EW3H7, EW11D4, EW11E4, and EW9C3 were provided by E. Ward, Ciba-Geigy, Inc.

Fig. 1C is a diagram showing the alignment of cosmid clones around the *RPS2* locus. Cosmid clones with the designation H are derivatives of the EW3H7 YAC clone whereas those with the designation E are derivatives of

the EW11E4 YAC clone. Vertical arrows indicate the relative positions of RFLP markers between the ecotypes La-*er* and the *rps2-101N* plant. The RFLP markers were identified by screening a Southern blot containing more than 50 different restriction enzyme digests using either the entire part or pieces of the corresponding cosmid clones as probes. The cosmid clones described in Fig. 1C were provided by J. Giraudat, C.N.R.S., Gif-sur-Yvette, France.

Figs. 1D and 1E are maps of *Eco*RI restriction endonuclease sites in the cosmids E4-4 and E4-6, respectively. The recombination break points surrounding the *RPS2* locus are located within the 4.5 and 7.5 kb *Eco*RI restriction endonuclease fragments.

Fig. 1F is a diagram showing the approximate location of genes which encode the RNA transcripts which have been identified by polyA$^+$ RNA blot analysis. The sizes of the transcripts are given in kilobase pairs below each transcript.

Fig. 2 is the complete nucleotide sequence of cDNA-4 comprising the *RPS2* [SEQ. ID. NO: 1] gene locus. The three reading frames are shown below the nucleotide sequence. The deduced amino acid sequence [SEQ. ID NOS:2-5] of reading frame "a" is provided and contains 909 amino acids. The methionine encoded by the ATG start codon is circled in open reading frame "a" of Fig. 2. The A of the ATG start codon is nucleotide 31 of Fig. 2.

Fig. 3 is the nucleotide sequence of the *avrRpt2* gene [SEQ. ID NO:105] and its deduced amino acid sequence [SEQ. ID NO:106]. A potential ribosome binding site is underlined. An inverted repeat is indicated by horizontal arrows at the 3′ end of the open reading frame. The deduced amino acid sequence is provided below the nucleotide sequence of the open reading frame.

Fig. 4 is a schematic summary of the complementation analysis that allowed functional confirmation that the DNA carried on p4104 and p4115 (encoding cDNA-4) confers *RPS2* disease resistance activity to *Arabidopsis thaliana* plants previously lacking *RPS2* disease resistance activity. Small vertical marks along the "genome" line represent restriction enzyme *Eco*RI recognition sites, and the numbers above this line represent the size, in kilobasepairs (kb), of the resulting DNA fragments (see also Fig. 1E). Opposite "cDNAs" are the approximate locations of the coding sequences for RNA transcripts (See also Fig. 1F); arrowheads indicate the direction of transcription for cDNAs 4, 5, and 6. For functional complementation experiments, *rps2-201C/rps2-201C* plants were genetically transformed with the *Arabidopsis thaliana* genomic DNA sequences indicated; these sequences were carried on the named plasmids (derivatives of the binary cosmid vector pSLJ4541) and delivered to the plant via *Agrobacterium*-mediated transformation methods. The disease resistance phenotype of the resulting transformants following inoculation with *P. syringae* expressing *avrRpt2* is given as "Sus." (susceptible, no resistance response) or "Res." (disease resistant).

The Genetic Basis for Resistance to Pathogens

An overview of the interaction between a plant host and a microbial pathogen is presented. The invasion of a plant by a potential pathogen can have a range of outcomes delineated by the following outcomes: either the pathogen successfully proliferates in the host, causing associated disease symptoms, or its growth is halted by the host defenses. In some plant-pathogen interactions, the visible hallmark of an active defense response is the so-called hypersensitive response or "HR". The HR

involves rapid necrosis of cells near the site of the infection and may include the formation of a visible dry brown lesion. Pathogens which elicit an HR on a given host are said to be avirulent on that host, the host is said to be resistant, and the plant-pathogen interaction is said to be incompatible. Strains which proliferate and cause disease on a particular host are said to be virulent; in this case the host is said to be susceptible, and the plant-pathogen interaction is said to be compatible

"Classical" genetic analysis has been used successfully to help elucidate the genetic basis of plant-pathogen recognition for those cases in which a series of strains (races) of a particular fungal or bacterial pathogen are either virulent or avirulent on a series of cultivars (or different wild accessions) of a particular host species. In many such cases, genetic analysis of both the host and the pathogen revealed that many avirulent fungal and bacterial strains differ from virulent ones by the possession of one or more avirulence (*avr*) genes that have corresponding "resistance" genes in the host. This avirulence gene-resistance gene correspondence is termed the "gene-for-gene" model (Crute, et al., (1985) pp 197-309 in: *Mechanisms of Resistance to Plant Disease*. R.S.S. Fraser, ed.; Ellingboe, (1981) Annu. Rev. Phytopathol. 19:125-143; Flor, (1971) Annu. Rev. Phytopathol. 9:275-296; Keen and Staskawicz, (1988) supra; and Keen et al. in: *Application of Biotechnology to Plant Pathogen Control*. I. Chet, ed., John Wiley & Sons, 1993, pp. 65-88). According to a simple formulation of this model, plant resistance genes encode specific receptors for molecular signals generated by *avr* genes. Signal transduction pathway(s) then carry the signal to a set of target genes that initiate the HR and other host defenses (Gabriel and Rolfe, (1990) Annu.

Rev. Phytopathol. 28:365-391). Despite this simple predictive model, the molecular basis of the *avr*-resistance gene interaction is still unknown.

One basic prediction of the gene-for-gene hypothesis has been convincingly confirmed at the molecular level by the cloning of a variety of bacterial *avr* genes (Innes, et al., (1993) J. Bacteriol. 175:4859-4869; Dong, et al., (1991) Plant Cell 3:61-72; Whelan et al., (1991) Plant Cell 3:49-59; Staskawicz et al., (1987) J. Bacteriol. 169:5789-5794; Gabriel et al., (1986) P.N.A.S., USA 83:6415-6419; Keen and Staskawicz, (1988) Annu. Rev. Microbiol. 42:421-440; Kobayashi et al., (1990) Mol. Plant-Microbe Interact. 3:94-102 and (1990) Mol. Plant-Microbe Interact. 3:103-111). Many of these cloned avirulence genes have been shown to correspond to individual resistance genes in the cognate host plants and have been shown to confer an avirulent phenotype when transferred to an otherwise virulent strain. The *avrRpt2* locus was isolated from *Pseudomonas syringae* pv. *tomato* and sequenced by Innes et al. (Innes, R. et al. (1993) J. Bacteriol. 175:4859-4869). Fig. 3 is the nucleotide sequence [SEQ. ID NO:105] and deduced amino acid sequence [SEQ. ID NO:6] of the *avrRpt2* gene.

Examples of known signals to which plants respond when infected by pathogens include harpins from *Erwinia* (Wei et al. (1992) Science 257:85-88) and *Pseudomonas* (He et al. (1993) Cell 73:1255-1266); *avr4* (Joosten et al. (1994) Nature 367:384-386) and avr9 peptides (van den Ackerveken et al (1992) Plant J. 2:359-366) from *Cladosporium*; *PopA*1 from *Pseudomonas* (Arlat et al. (1994) EMBO J. 13:543-553); avrD-generated lipopolysaccharide (Midland et al. (1993) J. Org. Chem. 58:2940-2945); and *NIP1* from *Rhynchosporium* (Hahn et al. (1993) Mol. Plant-Microbe Interact. 6:745-754).

Compared to *avr* genes, considerably less is known about plant resistance genes that correspond to specific *avr*-generated signals. The plant resistance gene, *RPS2* (rps for resistance to Pseudomonas syringae), the first gene of a new, previously unidentified class of plant disease resistance genes corresponds to a specific *avr* gene (*avrRpt2*). Some of the work leading up to the cloning of *RPS2* is described in Yu, et al., (1993), Molecular Plant-Microbe Interactions 6:434-443 and in Kunkel, et al., (1993) Plant Cell 5:865-875.

An apparently unrelated avirulence gene which corresponds specifically to plant disease resistance gene, *Pto*, has been isolated from tomato (*Lycopersicon esculentum*)(Martin et al., (1993) Science 262:1432-1436). Tomato plants expressing the *Pto* gene are resistant to infection by strains of *Pseudomonas syringae* pv. *tomato* that express the *avrPto* avirulence gene. The amino acid sequence inferred from the *Pto* gene DNA sequence displays strong similarity to serine-threonine protein kinases, implicating *Pto* in signal transduction. No similarity to the tomato *Pto* locus or any known protein kinases was observed for *RPS2*, suggesting that *RPS2* is representative of a new class of plant disease resistance genes.

The isolation of a race-specific resistance gene from *Zea mays* (corn) known as *Hm1* has been reported (Johal and Briggs (1992) Science 258:985-987). *Hm1* confers resistance against specific races of the fungal pathogen *Cochliobolus carbonum* by controlling degradation of a fungal toxin, a strategy that is mechanistically distinct from the avirulence-gene specific resistance of the *RPS2-avrRpt2* resistance mechanism.

The cloned *RPS2* gene of the invention can be used to facilitate the construction of plants that are resistant to specific pathogens and to overcome the inability to transfer disease resistance genes between

species using classical breeding techniques (Keen et al., (1993), supra). There now follows a description of the cloning and characterization of an *Arabidopsis thaliana* *RPS2* genetic locus, the *RPS2* genomic DNA, and the *RPS2* cDNA. The *avrRpt2* gene and the *RPS2* gene, as well as mutants *rps2-101C*, *rps2-102C*, and *rps2-201C* (also designated *rps2-201*), are described in Dong, et al., (1991) Plant Cell 3:61-72; Yu, et al., (1993) supra; Kunkel et al., (1993) supra; Whalen et al., (1991), supra; and Innes et al., (1993), supra). A mutant designated *rps2-101N* has also been isolated. The identification and cloning of the *RPS2* gene is described below.

RPS2 Overcomes Sensitivity to Pathogens Carrying the avrRpt2 Gene.

To demonstrate the genetic relationship between an avirulence gene in the pathogen and a resistance gene in the host, it was necessary first to isolate an avirulence gene. By screening *Pseudomonas* strains that are known pathogens of crop plants related to *Arabidopsis*, highly virulent strains, *P. syringae* pv. *maculicola (Psm)* ES4326, *P. syringae* pv. *tomato* (*Pst*) DC3000, and an avirulent strain, *Pst* MM1065 were identified and analyzed as to their respective abilities to grow in wild type *Arabidopsis thaliana* plants (Dong *et al.*, (1991) Plant Cell, 3:61-72; Whalen et al., (1991) Plant Cell 3:49-59; MM1065 is designated JL1065 in Whalen et al.). *Psm* ES4326 or *Pst* DC3000 can multiply 10^4 fold in *Arabidopsis thaliana* leaves and cause water-soaked lesions that appear over the course of two days. *Pst* MM1065 multiplies a maximum of 10 fold in *Arabidopsis thaliana* leaves and causes the appearance of a mildly chlorotic dry lesion after 48 hours. Thus, disease resistance is

associated with severely inhibited growth of the pathogen.

An avirulence gene (*avr*) of the *Pst* MM1065 strain was cloned using standard techniques as described in Dong *et al.* (1991), Plant Cell 3:61-72; Whalen et al., (1991) supra; and Innes et al., (1993), supra. The isolated avirulence gene from this strain was designated *avrRpt2*. Normally, the virulent strain *Psm* ES4326 or *Pst* DC3000 causes the appearance of disease symptoms after 48 hours as described above. In contrast, *Psm* ES4326/*avrRpt2* or *Pst* DC3000/*avrRpt2* elicits the appearance of a visible necrotic hypersensitivity response (HR) within 16 hours and multiplies 50 fold less than *Psm* ES4326 or *Pst* DC3000 in wild type *Arabidopsis thaliana* leaves (Dong *et al.*, (1991), supra; and Whalen et al., (1991), supra). Thus, disease resistance in a wild type *Arabidopsis* plant requires, in part, an avirulence gene in the pathogen or a signal generated by the avirulence gene.

The isolation of four *Arabidopsis thaliana* disease resistance mutants has been described using the cloned *avrRpt2* gene to search for the host gene required for disease resistance to pathogens carrying the *avrRpt2* gene (Yu et al., (1993), supra; Kunkel et al., (1993), supra). The four *Arabidopsis thaliana* mutants failed to develop an HR when infiltrated with *Psm* ES4326/*avrRpt2* or *Pst* DC3000/*avrRpt2* as expected for plants having lost their disease resistance capacity. In the case of one of these mutants, approximately 3000 five to six week old M_2 ecotype Columbia (Col-0 plants) plants generated by ethyl methanesulfonic acid (EMS) mutagenesis were hand-inoculated with *Psm* ES4326/*avrRpt2* and a single mutant, *rps2-101C,* was identified (resistance to Pseudomonas syringae) (Yu *et al.*, (1993), supra).

The second mutant was isolated using a procedure that specifically enriches for mutants unable to mount an

HR (Yu *et al.*, (1993), supra). When 10-day old *Arabidopsis thaliana* seedlings growing on petri plates are infiltrated with *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) NPS3121 versus *Psp* NPS3121/*avrRpt2*, about 90% of the plants infiltrated with *Psp* NPS3121 survive, whereas about 90%-95% of the plants infiltrated with *Psp* NPS3121/*avrRpt2* die. Apparently, vacuum infiltration of an entire small *Arabidopsis thaliana* seedling with *Psp* NPS3121/*avrRpt2* elicits a systemic HR which usually kills the seedling. In contrast, seedlings infiltrated with *Psp* NPS3121 survive because *Psp* NPS3121 is a weak pathogen on *Arabidopsis thaliana*. The second disease resistance mutant was isolated by infiltrating 4000 EMS-mutagenized Columbia M_2 seedlings with *Psp* NPS3121/*avrRpt2*. Two hundred survivors were obtained. These were transplanted to soil and re-screened by hand inoculation when the plants reached maturity. Of these 200 survivors, one plant failed to give an HR when hand-infiltrated with *Psm* ES4326/*avrRpt2*. This mutant was designated *rps2-102C* (Yu *et al.*, (1993), supra).

A third mutant, *rps2-201C*, was isolated in a screen of approximately 7500 M_2 plants derived from seed of *Arabidopsis thaliana* ecotype Col-O that had been mutagenized with diepoxybutane (Kunkel et al., (1993), supra). Plants were inoculated by dipping entire leaf rosettes into a solution containing *Pst* DC3000/*avrRpt2* bacteria and the surfactant Silwet L-77 (Whalen et al., (1991), supra), incubating plants in a controlled environment growth chamber for three to four days, and then visually observing disease symptom development. This screen revealed four mutant lines (carrying the *rps2-201C*, *rps2-202C*, *rps2-203C*, and *rps2-204C* alleles), and plants homozygous for *rps2-201C* were a primary subject for further study (Kunkel et al., (1993), supra and the instant application).

Isolation of the fourth *rps2* mutant, *rps2-101N*, has not yet been published. This fourth isolate is either a mutant or a susceptible *Arabidopsis* ecotype. Seeds of the *Arabidopsis* Nossen ecotype were gamma-irradiated and then sown densely in flats and allowed to germinate and grow through a nylon mesh. When the plants were five to six weeks old, the flats were inverted, the plants were partially submerged in a tray containing a culture of *Psm* ES4326/*avrRpt2*, and the plants were vacuum infiltrated in a vacuum desiccator. Plants inoculated this way develop an HR within 24 hours. Using this procedure, approximately 40,000 plants were screened and one susceptible plant was identified. Subsequent RFLP analysis of this plant suggested that it may not be a Nossen mutant but rather a different *Arabidopsis* ecotype that is susceptible to *Psm* ES4326/*avrRpt2*. This plant is referred to as *rps2-101N*. The isolated mutants *rps2-101C*, *rps2-102C*, *rps2-201C*, and *rps2-101N* are referred to collectively as the "*rps2* mutants".

<u>The *rps2* Mutants Fail to Specifically Respond to the Cloned Avirulence Gene, *avrRpt2*</u>.

The *RPS2* gene product is specifically required for resistance to pathogens carrying the avirulence gene, *avrRpt2*. A mutation in Rps2 polypeptide that eliminates or reduces its function would be observable as the absence of a hypersensitive response upon infiltration of the pathogen. The *rps2* mutants displayed disease symptoms or a null response when infiltrated with *Psm* ES4326/*avrRpt2*, *Pst* DC3000/*avrRpt2* or *Psp* NPS3121/*avrRpt2*, respectively. Specifically, no HR response was elicited, indicating that the plants were susceptible and had lost resistance to the pathogen despite the presence of the *avrRpt2* gene in the pathogen.

Pathogen growth in *rps2* mutant plant leaves was similar in the presence and absence of the *avrRpt2* gene. *Psm* ES4326 and *Psm* ES4326/*avrRpt2* growth in *rps2* mutants was compared and found to multiply equally well in the *rps2* mutants, at the same rate that *Psm* Es4326 multiplied in wild-type *Arabidopsis* leaves. Similar results were observed for *Pst* DC3000 and *Pst* DC3000/*avrRpt2* growth in *rps2* mutants.

The *rps2* mutants displayed a HR when infiltrated with *Pseudomonas* pathogens carrying other *avr* genes, *Psm* ES4326/*avrB*, *Pst* DC3000/*avrB*, *Psm* ES4326/*avrRpm1*, *Pst* DC3000/*avrRpm1*. The ability to mount an HR to an *avr* gene other than *avrRpt2* indicates that the *rps2* mutants isolated by selection with *avrRpt2* are specific to *avrRpt2*.

Mapping and Cloning of the *RPS2* Gene.

Genetic analysis of *rps2* mutants *rps2-101C*, *rps2-102C*, *rps-201C* and *rps-101N* showed that they all corresponded to genes that segregated as expected for a single Mendelian locus and that all four were most likely allelic. The four *rps2* mutants were mapped to the bottom of chromosome IV using standard RFLP mapping procedures including polymerase chain reaction (PCR)-based markers (Yu *et al.*, (1993), supra; Kunkel *et al.*, (1993), supra; and Mindrinos, M., unpublished). Segregation analysis showed that *rps2-101C* and *rps2-102C* are tightly linked to the PCR marker, PG11, while the RFLP marker M600 was used to define the chromosome location of the *rps2-201C* mutation (Fig. 1A) (Yu *et al.*, (1993), supra; Kunkel *et al.*, (1993), supra). *RPS2* has subsequently been mapped to the centromeric side of PG11.

Heterozygous *RPS2*/*rps2* plants display a defense response that is intermediate between those displayed by the wild-type and homozygous *rps2*/*rps2* mutant plants (Yu,

et al., (1993), supra; and Kunkel et al., (1993), supra). The heterozygous plants mounted an HR in response to *Psm* ES4326/*avrRpt2* or *Pst* DC3000/*avrRpt2* infiltration; however, the HR appeared later than in wild type plants and required a higher minimum inoculum (Yu, et al., (1993), supra; and Kunkel et al., (1993), supra).

High Resolution Mapping of the *RPS2* Gene and *RPS2* cDNA Isolation.

To carry out map-based cloning of the *RPS2* gene, *rps2-101N/rps2-101N* was crossed with Landsberg *erecta* *RPS2/RPS2*. Plants of the F_1 generation were allowed to self pollinate (to "self") and 165 F_2 plants were selfed to generate F_3 families. Standard RFLP mapping procedures showed that *rps2-101N* maps close to and on the centromeric side of the RFLP marker, PG11. To obtain a more detailed map position, *rps2-101N/rps-101N* was crossed with a doubly marked Landsberg *erecta* strain containing the recessive mutations, *cer2* and *ap2*. The genetic distance between *cer2* and *ap2* is approximately 15 cM, and the *rps2* locus is located within this interval. F_2 plants that displayed either a *CER2 ap2* or a *cer2 AP2* genotype were collected, selfed, and scored for *RPS2* by inoculating at least 20 F_3 plants for each F_2 with *Psm* ES4326/*avrRpt2*. DNA was also prepared from a pool of approximately 20 F_3 plants for each F_2 line. The *CER2 ap2* and *cer2 AP2* recombinants were used to carry out a chromosome walk that is illustrated in Figure 1.

As shown in Figure 1, *RPS2* was mapped to a 28-35 kb region spanned by cosmid clones E4-4 and E4-6. This region contains at least six genes that produce detectable transcripts. There were no significant differences in the sizes of the transcripts or their level of expression in the *rps2* mutants as determined by RNA blot analysis. cDNA clones of each of these

transcripts were isolated and five of these were sequenced. As is described below, one of these transcripts, cDNA-4, was shown to correspond to the *RPS2* locus. From this study, three independent cDNA clones (cDNA-4-4, cDNA-4-5, and cDNA-4-11) were obtained corresponding to *RPS2* from Columbia ecotype wild type plants. The apparent sizes of *RPS2* transcripts were 3.8 and 3.1 kb as determined by RNA blot analysis.

A fourth independent cDNA-4 clone (cDNA-4-2453) was obtained using map-based isolation of *RPS2* in a separate study. Yeast artificial chromosome (YAC) clones were identified that carry contiguous, overlapping inserts of *Arabidopsis thaliana* ecotype Col-O genomic DNA from the M600 region spanning approximately 900 kb in the *RPS2* region. *Arabidopsis* YAC libraries were obtained from J. Ecker and E. Ward, supra and from E. Grill (Grill and Somerville (1991) Mol. Gen. Genet. 226:484-490). Cosmids designated "H" and "E" were derived from the YAC inserts and were used in the isolation of *RPS2* (Fig. 1).

The genetic and physical location of *RPS2* was more precisely defined using physically mapped RFLP, RAPD (random amplified polymorphic DNA) and CAPS (cleaved amplified polymorphic sequence) markers. Segregating populations from crosses between plants of genotype *RPS2/RPS2* (No-O wild type) and *rps2-201/rps2-201* (Col-O background) were used for genetic mapping. The *RPS2* locus was mapped using markers 17B7LE, PG11, M600 and other markers. For high-resolution genetic mapping, a set of tightly linked RFLP markers was generated using insert end fragments from YAC and cosmid clones (Fig. 1) (Kunkel et al. (1993), supra; Konieczny and Ausubel (1993) Plant J. 4:403-410; and Chang et al. (1988) PNAS USA 85:6856-6860). Cosmid clones E4-4 and E4-6 were then used to identify expressed transcripts (designated cDNA-

4, -5, -6, -7, -8 of Fig 1F) from this region, including the cDNA-4-2453 clone.

RPS2 DNA Sequence Analysis.

DNA sequence analysis of cDNA-4 from wild-type Col-O plants and from mutants *rps2-101C*, *rps2-102C*, *rps2-201C* and *rps2-101N* showed that cDNA-4 corresponds to *RPS2*. DNA sequence analysis of *rps2-101C*, *rps2-102C* and *rps2-201C* revealed changes from the wild-type sequence as shown in Table 1. The numbering system in Table 1 starts at the ATG start codon encoding the first methionine where A is nucleotide 1. DNA sequence analysis of cDNA-4 corresponding to mutant *rps2-102C* showed that it differed from the wild type sequence at amino acid residue 476. Moreover, DNA sequence analysis of the cDNA corresponding to cDNA-4 from *rps2-101N* showed that it contained a 10 bp insertion at amino acid residue 581, a site within the leucine-rich repeat region which causes a shift in the *RPS2* reading frame. Mutant *rps2-101C* contains a mutation that leads to the formation of a chain termination codon. The DNA sequence of mutant allele *rps2-201C* revealed a mutation altering a single amino acid within a segment of the LRR region that also has similarity to the helix-loop-helix motif, further supporting the designation of this locus as the *RPS2* gene. The DNA and amino acid sequences are shown in Figure 2 [SEQ. ID NO:1 and SEQ ID NOS:2-5, respectively].

Table 1.

Mutant	Wild type	position of	Change mutation
rps2-101C	703 TGA 705	704	TAA Stop Codon
rps2-101N	1741 GTG 1743	1741	GTGGAGTTGTATG Insertion
rps2-102C 476	1426 AGA 1428 arg	1427	AAA Amino acid lys
rps2-201C	2002 ACC 2004 thr	2002	CCC Amino acid pro

DNA sequence analysis of cDNA-4 corresponding to *RPS2* from wild-type Col-O plants revealed an open reading frame (between two stop codons) spanning 2,751 bp. There are 2,727 bp between the first methionine codon of this reading frame and the 3'-stop codon, which corresponds to a deduced 909 amino acid polypeptide (See open reading frame "a" of Fig. 2). The amino acid sequence has a relative molecular weight of 104,460 and a pI of 6.51.

RPS2 belongs to a new class of disease resistance genes; the structure of the Rps2 polypeptide does not resemble the protein structure of the product of the only previously cloned and publicized avirulence gene-specific plant disease resistance gene, *Pto*, which has a putative protein kinase domain. From the above analysis of the deduced amino acid sequence, *RPS2* contains several distinct protein domains conserved in other proteins from both eukaryotes and prokaryotes. These domains include but are not limited to Leucine Rich Repeats (LRR) (Kobe and Deisenhofer, (1994) Nature 366:751-756); P-loop (Saraste et al. (1990) Trends in Biological Sciences TIBS 15:430-434; Helix-Loop-Helix (Murre et al. (1989) Cell 56:777-783; and Leucine Zipper (Rodrigues and Park (1993) Mol. Cell Biol. 13:6711-6722). The amino acid sequence

of Rps2 contains a LRR motif (LRR motif from amino acid residue 505 to amino acid residue 867), which is present in many known proteins and which is thought to be involved in protein-protein interactions and may thus allow interaction with other proteins that are involved in plant disease resistance. The N-terminal portion of the Rps2 polypeptide LRR is, for example, related to the LRR of yeast (*Saccharomyces cerevisiae*) adenylate cyclase, CYR1. A region predicted to be a transmembrane spanning domain (Klein et al. (1985) Biochim., Biophys. Acta 815:468-476) is located from amino acid residue 350 to amino acid residue 365, N-terminal to the LRR. An ATP/GTP binding site motif (P-loop) is predicted to be located between amino acid residue 177 and amino acid residue 194, inclusive.

From the above analysis of the deduced amino acid sequence, the Rps2 polypeptide may have a membrane-receptor structure which consists of an N-terminal extracellular region and a C-terminal cytoplasmic region. Alternatively, the topology of the Rps2 may be the opposite: an N-terminal cytoplasmic region and a C-terminal extracellular region. LRR motifs are extracellular in many cases and the Rps2 LRR contains five potential N-glycosylation sites.

Identification of *RPS2* by Functional Complementation.

Complementation of *rps2-201* homozygotes with genomic DNA corresponding to *Arabidopsis thaliana* functionally confirmed that the genomic region encoding cDNA-4 carries *RPS2* activity. Cosmids were constructed that contained overlapping contiguous sequences of wild type *Arabidopsis thaliana* DNA from the *RPS2* region contained in YACs EW11D4, EW9C3, and YUP11F1 of Fig. 1 and Fig. 4. The cosmid vectors were constructed from pSLJ4541 (obtained from J. Jones, Sainsbury Institute,

Norwich, England) which contains sequences that allow the inserted sequence to be integrated into the plant genome via *Agrobacterium*-mediated transformation (designated "binary cosmid"). "H" and "E" cosmids (Fig. 1) were used to identify clones carrying DNA from the *Arabidopsis thaliana* genomic *RPS2* region.

More than forty binary cosmids containing inserted *RPS2* region DNA were used to transform *rps2-201* homozygous mutants utilizing *Agrobacterium*-mediated transformation (Chang et al. ((1990) p. 28, Abstracts of the Fourth International Conference on *Arabidopsis* Research, Vienna, Austria). Transformants which remained susceptible (determined by methods including the observed absence of an HR following infection to *P. syringae* pv. *phaseolicola* strain 3121 carrying *avrRpt2* and Psp 3121 without *avrRpt2*) indicated that the inserted DNA did not contain functional *RPS2*. These cosmids conferred the "Sus." or susceptible phenotype indicated in Fig. 4. Transformants which had aquired *avrRpt2*-specific disease resistance (determined by methods including the display of a strong hypersensitive response (HR) when inoculated with *Psp* 3121 with *avrRpt2*, but not following inoculation with *Psp* 3121 without *avrRpt2*) suggested that the inserted DNA contained a functional *RPS2* gene capable of conferring the "Res." or resistant phenotype indicated in Fig. 4. Transformants obtained using the pD4 binary cosmid displayed a strong resistance phenotype as described above. The presence of the insert DNA in the transformants was confirmed by classical genetic analysis (the tight genetic linkage of the disease resistance phenotype and the kanamycin resistance phenotype conferred by the cotransformed selectable marker) and Southern analysis. These results indicated that *RPS2* is encoded by a segment of the 18 kb *Arabidopsis thaliana* genomic region carried on cosmid pD4 (Fig. 4).

To further localize the *RPS2* locus and confirm its ability to confer a resistance phenotype on the *rps2-201* homozygous mutants, a set of six binary cosmids containing partially overlapping genomic DNA inserts were tested. The overlapping inserts pD2, pD4, pD14, pD15, pD27, and pD47 were chosen based on the location of the transcription corresponding to the five cDNA clones in the *RPS2* region (Fig. 4). These transformation experiments utilized a vacuum infiltration procedure (Bechtold et al. (1993) C.R. Acad. Sci. Paris 316:1194-1199) for *Agrobacterium*-mediated transformation. *Agrobacterium*-mediated transformations with cosmids pD2, pD14, pD15, pD39, and pD46 were performed using a root transformation/regeneration protocol (Valveekens et al. (1988), PNAS 85:5536-5540). The results of pathogen inoculation experiments assaying for *RPS2* activity in these transformants is indicated in Fig. 4.

Additional transformation experiments utilized binary cosmids carrying the complete coding region and more than 1 kb of upstream genomic sequence for only cDNA-4 or cDNA-6. Using the vacuum infiltration transformation method, three independent transformants were obtained that carried the wild-type cDNA-6 genomic region in a *rps2-201c* homozygous background (pAD431 of Fig. 4). None of these plants displayed *avrRpt2*-dependent disease resistance. Homozygous *rps2-201c* mutants were transformed with wild-type genomic cDNA-4 (p4104 and p4115, each carrying Col-0 genomic sequences corresponding to all of the cDNA-4 open reading frame, plus approximately 1.7 kb of 5′ upstream sequence and approximately 0.3 kb of 3′ sequence downstream of the stop codon). These p4104 and p4115 transformants displayed a disease resistance phenotype similar to the wild-type *RPS2* homozygotes from which the *rps2* were derived. Additional mutants (*rps2-101N* and *rps2-101C*

homozygotes) also displayed *avrRpt2*-dependent resistance when transformed with the cDNA-4 genomic region.

RPS2 Sequences Allow Detection of Other Resistance Genes.

DNA blot analysis of *Arabidopsis thaliana* genomic DNA using *RPS2* cDNA as the probe showed that *Arabidopsis* contains several DNA sequences that hybridize to *RPS2* or a portion thereof, suggesting that there are several related genes in the *Arabidopsis* genome.

From the aforementioned description and the nucleic acid sequence [SEQ. ID. NO:1] shown in Fig. 2, it is possible to isolate other plant disease resistance genes having about 50% or greater sequence identity to the *RPS2* gene. Detection and isolation can be carried out with an oligonucleotide probe containing the *RPS2* gene or a portion thereof greater than about 18 nucleic acids in length. Probes to sequences encoding specific structural features of the Rps2 polypeptide [SEQ. ID NOS:2-5] are preferred as they provide a means of isolating disease resistance genes having similar structural domains. Hybridization can be done using standard techniques such as are described in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, (1989).

For example, high stringency conditions for detecting the *RPS2* gene include hybridization at about 42°C, and about 50% formamide; a first wash at about 65°C, about 2X SSC, and 1% SDS; followed by a second wash at about 65°C and about 0.1% SSC. Lower stringency conditions for detecting *RPS* genes having about 50% sequence identity to the *RPS2* gene are detected by, for example, hybridization at about 42°C in the absence of formamide; a first wash at about 42°C, about 6X SSC, and about 1% SDS; and a second wash at about 50°C, about 6X SSC, and about 1% SDS. An approximately 350 nucleotide

DNA probe encoding the middle portion of the LRR region of Rps2 was used as a probe in the above example. Under lower stringency conditions, a minimum of 5 DNA bands were detected in *Bam*HI digested *Arabidopsis thaliana* genomic DNA as sequences having sufficient sequence identity to hybridize to DNA encoding the middle portion of the LRR motif of Rps2. Similar results were obtained using a probe containing a 300 nucleotide portion of the *RPS2* gene encoding the extreme N-terminus of Rps2 outside of the LRR motif.

Isolation of other disease resistance genes is performed by PCR amplification techniques well known to those skilled in the art of molecular biology using oligonucleotide primers designed to amplify only sequences flanked by the oligonucleotides in genes having sequence identity to *RPS2*. The primers are optionally designed to allow cloning of the amplified product into a suitable vector.

RPS2 Expression in Transgenic Plant Cells and Plants

The expression of the *RPS2* gene in plants susceptible to pathogens carrying *avrRpt2* is achieved by introducing into a plant a DNA sequence containing the *RPS2* gene for expression of the Rps2 polypeptide. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants are available to the public; such vectors are described in, e.g., Pouwels et al., *Cloning Vectors: A Laboratory Manual*, 1985, Supp. 1987); Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989; and Gelvin et al., *Plant Molecular Biology Manual*, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include (1) one or more cloned plant genes under the transcriptional control of 5′ and 3′ regulatory sequences and (2) a dominant selectable

marker. Such plant expression vectors may also contain, if desired, a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

An example of a useful plant promoter which could be used to express a plant resistance gene according to the invention is a caulimovirus promoter, e.g., the cauliflower mosaic virus (CaMV) 35S promoter. These promoters confer high levels of expression in most plant tissues, and the activity of these promoters is not dependent on virally encoded proteins. CaMV is a source for both the 35S and 19S promoters. In most tissues of transgenic plants, the CaMV 35S promoter is a strong promoter (see, e.g., Odel et al., Nature 313:810, (1985)). The CaMV promoter is also highly active in monocots (see, e.g., Dekeyser et al., Plant Cell 2:591, (1990); Terada and Shimamoto, Mol. Gen. Genet. 220:389, (1990)).

Other useful plant promoters include, without limitation, the nopaline synthase promoter (An et al., Plant Physiol. 88:547, (1988)) and the octopine synthase promoter (Fromm et al., Plant Cell 1:977, (1989)).

For certain applications, it may be desirable to produce the *RPS2* gene product or the *avrRpt2* gene product in an appropriate tissue, at an appropriate level, or at an appropriate developmental time. Thus, there are a variety of gene promoters, each with its own distinct characteristics embodied in its regulatory sequences, shown to be regulated in response to the environment, hormones, and/or developmental cues. These include gene promoters that are responsible for (1) heat-regulated

gene expression (see, e.g., Callis et al., Plant Physiol. 88: 965, (1988)), (2) light-regulated gene expression (e.g., the pea *rbcS-3A* described by Kuhlemeier et al., Plant Cell 1: 471, (1989); the maize *rbcS* promoter described by Schaffner and Sheen, Plant Cell 3: 997, (1991); or the cholorphyll a/b-binding protein gene found in pea described by Simpson et al., EMBO J. 4: 2723, (1985)), (3) hormone-regulated gene expression (e.g., the abscisic acid responsive sequences from the *Em* gene of wheat described Marcotte et al., Plant Cell 1:969, (1989)), (4) wound-induced gene expression (e.g., of *wunI* described by Siebertz et al., Plant Cell 1: 961, (1989)), or (5) organ-specific gene expression (e.g., of the tuber-specific storage protein gene described by Roshal et al., EMBO J. 6:1155, (1987); the 23-kDa zein gene from maize described by Schernthaner et al., EMBO J. 7: 1249, (1988); or the French bean ß-phaseolin gene described by Bustos et al., Plant Cell 1:839, (1989)).

Plant expression vectors may also optionally include RNA processing signals, e.g, introns, which have been shown to be important for efficient RNA synthesis and accumulation (Callis et al., Genes and Dev. 1: 1183, (1987)). The location of the RNA splice sequences can influence the level of transgene expression in plants. In view of this fact, an intron may be positioned upstream or downstream of an Rps2 polypeptide-encoding sequence in the transgene to modulate levels of gene expression.

In addition to the aforementioned 5′ regulatory control sequences, the expression vectors may also include regulatory control regions which are generally present in the 3′ regions of plant genes (Thornburg et al., Proc. Natl Acad. Sci USA 84: 744, (1987); An et al., Plant Cell 1: 115, (1989)). For example, the 3′ terminator region may be included in the expression

vector to increase stability of the mRNA. One such terminator region may be derived from the PI-II terminator region of potato. In addition, other commonly used terminators are derived from the octopine or nopaline synthase signals.

The plant expression vector also typically contains a dominant selectable marker gene used to identify the cells that have become transformed. Useful selectable marker genes for plant systems include genes encoding antibiotic resistance genes, for example, those encoding resistance to hygromycin, kanamycin, bleomycin, G418, streptomycin or spectinomycin. Genes required for photosynthesis may also be used as selectable markers in photosynthetic-deficient strains. Finally, genes encoding herbicide resistance may be used as selectable markers; useful herbicide resistance genes include the *bar* gene encoding the enzyme phosphinothricin acetyltransferase, which confers resistance to the broad spectrum herbicide Basta® (Hoechst AG, Frankfurt, Germany).

Efficient use of selectable markers is facilitated by a determination of the susceptibility of a plant cell to a particular selectable agent and a determination of the concentration of this agent which effectively kills most, if not all, of the transformed cells. Some useful concentrations of antibiotics for tobacco transformation include, e.g., 75-100 μg/ml (kanamycin), 20-50 μg/ml (hygromycin), or 5-10 μg/ml (bleomycin). A useful strategy for selection of transformants for herbicide resistance is described, e.g., in Vasil I.K., *Cell Culture and Somatic Cell Genetics of Plants*, Vol I, II, III Laboratory Procedures and Their Applications Academic Press, New York, 1984.

It should be readily apparent to one skilled in the field of plant molecular biology that the level of

gene expression is dependent not only on the combination of promoters, RNA processing signals and terminator elements, but also on how these elements are used to increase the levels of gene expression.

Plant Transformation

Upon construction of the plant expression vector, several standard methods are known for introduction of the recombinant genetic material into the host plant for the generation of a transgenic plant. These methods include (1) Agrobacterium-mediated transformation (*A. tumefaciens* or *A. rhizogenes*) (see, e.g., Lichtenstein and Fuller In: *Genetic Engineering*, vol 6, PWJ Rigby, ed, London, Academic Press, 1987; and Lichtenstein, C.P., and Draper, J,. In: *DNA Cloning*, Vol II, D.M. Glover, ed, Oxford, IRI Press, 1985), (2) the particle delivery system (see, e.g., Gordon-Kamm et al., Plant Cell 2:603, (1990); or BioRad Technical Bulletin 1687, *supra*), (3) microinjection protocols (see, e.g., Green et al., *Plant Tissue and Cell Culture*, Academic Press, New York, 1987), (4) polyethylene glycol (PEG) procedures (see, e.g., Draper et al., Plant Cell Physiol 23:451, (1982); or e.g., Zhang and Wu, Theor. Appl. Genet. 76:835, (1988)), (5) liposome-mediated DNA uptake (see, e.g., Freeman et al., Plant Cell Physiol 25: 1353, (1984)), (6) electroporation protocols (see, e.g., Gelvin et al supra; Dekeyser et al. supra; or Fromm et al Nature 319: 791, (1986)), and (7) the vortexing method (see, e.g., Kindle, K., Proc. Natl. Acad. Sci., USA 87:1228, (1990)).

The following is an example outlining an *Agrobacterium*-mediated plant transformation. The general process for manipulating genes to be transferred into the genome of plant cells is carried out in two phases. First, all the cloning and DNA modification steps are done in *E. coli*, and the plasmid containing the gene

construct of interest is transferred by conjugation into *Agrobacterium*. Second, the resulting *Agrobacterium* strain is used to transform plant cells. Thus, for the generalized plant expression vector, the plasmid contains an origin of replication that allows it to replicate in *Agrobacterium* and a high copy number origin of replication functional in *E. coli*. This permits facile production and testing of transgenes in *E.coli* prior to transfer to *Agrobacterium* for subsequent introduction into plants. Resistance genes can be carried on the vector, one for selection in bacteria, e.g., streptomycin, and the other that will express in plants, e.g., a gene encoding for kanamycin resistance or an herbicide resistance gene. Also present are restriction endonuclease sites for the addition of one or more transgenes operably linked to appropriate regulatory sequences and directional T-DNA border sequences which, when recognized by the transfer functions of *Agrobacterium*, delimit the region that will be transferred to the plant.

In another example, plant cells may be transformed by shooting into the cell tungsten microprojectiles on which cloned DNA is precipitated. In the Biolistic Apparatus (Bio-Rad, Hercules, CA) used for the shooting, a gunpowder charge (22 caliber Power Piston Tool Charge) or an air-driven blast drives a plastic macroprojectile through a gun barrel. An aliquot of a suspension of tungsten particles on which DNA has been precipitated is placed on the front of the plastic macroprojectile. The latter is fired at an acrylic stopping plate that has a hole through it that is too small for the macroprojectile to go through. As a result, the plastic macroprojectile smashes against the stopping plate and the tungsten microprojectiles continue toward their target through the hole in the plate. For the instant invention the target

can be any plant cell, tissue, seed, or embryo. The DNA introduced into the cell on the microprojectiles becomes integrated into either the nucleus or the chloroplast.

Transfer and expression of transgenes in plant cells is now routine practice to those skilled in the art. It has become a major tool to carry out gene expression studies and to attempt to obtain improved plant varieties of agricultural or commercial interest.

Transgenic Plant Regeneration

Plant cells transformed with a plant expression vector can be regenerated, e.g., from single cells, callus tissue or leaf discs according to standard plant tissue culture techniques. It is well known in the art that various cells, tissues and organs from almost any plant can be successfully cultured to regenerate an entire plant; such techniques are described, e.g., in Vasil supra; Green et al., supra; Weissbach and Weissbach, supra; and Gelvin et al., supra.

In one possible example, a vector carrying a selectable marker gene (e.g., kanamycin resistance), a cloned *RPS2* gene under the control of its own promoter and terminator or, if desired, under the control of exogenous regulatory sequences such as the 35S CaMV promoter and the nopaline synthase terminator is transformed into *Agrobacterium*. Transformation of leaf tissue with vector-containing *Agrobacterium* is carried out as described by Horsch et al. (Science 227: 1229, (1985)). Putative transformants are selected after a few weeks (e.g., 3 to 5 weeks) on plant tissue culture media containing kanamycin (e.g. 100 μg/ml). Kanamycin-resistant shoots are then placed on plant tissue culture media without hormones for root initiation. Kanamycin-resistant plants are then selected for greenhouse growth. If desired, seeds from self-fertilized transgenic plants

can then be sowed in a soil-less media and grown in a greenhouse. Kanamycin-resistant progeny are selected by sowing surfaced sterilized seeds on hormone-free kanamycin-containing media. Analysis for the integration of the transgene is accomplished by standard techniques (see, e.g., Ausubel et al. supra; Gelvin et al. supra).

Transgenic plants expressing the selectable marker are then screened for transmission of the transgene DNA by standard immunoblot and DNA and RNA detection techniques. Each positive transgenic plant and its transgenic progeny are unique in comparison to other transgenic plants established with the same transgene. Integration of the transgene DNA into the plant genomic DNA is in most cases random and the site of integration can profoundly effect the levels, and the tissue and developmental patterns of transgene expression. Consequently, a number of transgenic lines are usually screened for each transgene to identify and select plants with the most appropriate expression profiles.

Transgenic lines are evaluated for levels of transgene expression. Expression at the RNA level is determined initially to identify and quantitate expression-positive plants. Standard techniques for RNA analysis are employed and include PCR amplification assays using oligonucleotide primers designed to amplify only transgene RNA templates and solution hybridization assays using transgene-specific probes (see, e.g., Ausubel et al., supra). The RNA-positive plants are then analyzed for protein expression by Western immunoblot analysis using Rps2 polypeptide-specific antibodies (see, e.g., Ausubel et al., supra). In addition, *in situ* hybridization and immunocytochemistry according to standard protocols can be done using transgene-specific nucleotide probes and antibodies, respectively, to localize sites of expression within transgenic tissue.

Once the Rps2 polypeptide has been expressed in any cell or in a transgenic plant (e.g., as described above), it can be isolated using any standard technique, e.g., affinity chromatography. In one example, an anti-Rps2 antibody (e.g., produced as described in Ausubel et al., supra, or by any standard technique) may be attached to a column and used to isolate the polypeptide. Lysis and fractionation of Rps2-producing cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra). Once isolated, the recombinant polypeptide can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, Work and Burdon, eds., Elsevier, 1980).

These general techniques of polypeptide expression and purification can also be used to produce and isolate useful Rps2 fragments or analogs.

Use

Introduction of *RPS2* into a transformed plant cell provides for resistance to bacterial pathogens carrying the *avrRpt2* avirulence gene. For example, transgenic plants of the instant invention expressing *RPS2* might be used to alter, simply and inexpensively, the disease resistance of plants normally susceptible to plant pathogens carrying the avirulence gene, *avrRpt2*.

The invention also provides for broad-spectrum pathogen resistance by mimicking the natural mechanism of host resistance. First, the *RPS2* transgene is expressed in plant cells at a sufficiently high level to initiate the plant defense response constitutively in the absence of signals from the pathogen. The level of expression associated with plant defense response initiation is determined by measuring the levels of defense response

gene expression as described in Dong et al., supra. Second, the *RPS2* transgene is expressed by a controllable promoter such as a tissue-specific promoter, cell-type specific promoter or by a promoter that is induced by an external signal or agent thus limiting the temporal and tissue expression of a defense response. Finally, the *RPS2* gene product is co-expressed with the *avrRpt2* gene product. The *RPS2* gene is expressed by its natural promoter, by a constitutively expressed promoter such as the CaMV 35S promoter, by a tissue-specific or cell-type specific promoter, or by a promoter that is activated by an external signal or agent. Co-expression of *RPS2* and *avrRpt2* will mimic the production of gene products associated with the initiation of the plant defense response and provide resistance to pathogens in the absence of specific resistance gene-avirulence gene corresponding pairs in the host plant and pathogen.

The invention also provides for expression in plant cells of a nucleic acid having the sequence [SEQ. ID. NO:1] of Fig. 2 or the expression of a degenerate variant thereof encoding the amino acid sequence [SEQ. ID NOS:2-5] of open reading frame "a" of Fig. 2.

The invention further provides for the isolation of nucleic acid sequences having about 50% or greater sequence identity to *RPS2* by using the *RPS2* sequence [SEQ. ID. NO:1] of Fig. 2 or a portion thereof greater than about 18 nucleic acids in length as a probe. Appropriate reduced hybridization stringency conditions are utilized to isolate DNA sequences having about 50% or greater sequence identity to the *RPS2* sequence [SEQ. ID. NO: 1] of Fig. 2.

The invention will provide disease resistance to plants, especially crop plants, most especially important crop plants such as tomato, pepper, maize, wheat, rice and legumes such as soybean and bean, or any plant which

is susceptible to pathogens carrying an avirulence gene, e.g., the *avrRpt2* avirulence gene. Such pathogens include, but are not limited to, *Pseudomonas syringae* strains.

The invention also includes any biologically active fragment or analog of an Rps2 polypeptide. By "biologically active" is meant possessing any in vivo activity which is characteristic of the Rps2 polypeptide [SEQ. ID NOS:2-5] shown in Fig. 2. A useful Rps2 fragment or Rps2 analog is one which exhibits a biological activity in any biological assay for disease resistance gene product activity, for example, those assays described by Dong et al. (1991), supra; Yu et al. (1993) supra; and Kunkel et al. (1993) supra; and Whalen et al. (1991). In particular, a biologically active Rps2 polypeptide fragment or analog is capable of providing substantial resistance to plant pathogens carrying the *avrRpt2* avirulence gene. By substantial resistance is meant at least partial reduction in susceptibility to plant pathogens carrying the *avrRpt2* gene.

Preferred analogs include Rps2 polypeptides (or biologically active fragments thereof) whose sequences differ from the wild-type sequence only by conservative amino acid substitutions, for example, substitution of one amino acid for another with similar characteristics (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish the polypeptide's biological activity.

Analogs can differ from naturally occurring Rps2 polypeptide in amino acid sequence or can be modified in ways that do not involve sequence, or both. Analogs of the invention will generally exhibit at least 70%, preferably 80%, more preferably 90%, and most preferably 95% or even 99%, homology with a segment of 20 amino acid

residues, preferably 40 amino acid residues, or more preferably the entire sequence of a naturally occurring Rps2 polypeptide sequence [SEQ. ID NOS:2-5].

Alterations in primary sequence include genetic variants, both natural and induced. Also included are analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids. Also included in the invention are Rps2 polypeptides modified by *in vivo* chemical derivatization of polypeptides, including acetylation, methylation, phosphorylation, carboxylation, or glycosylation.

In addition to substantially full-length polypeptides, the invention also includes biologically active fragments of the polypeptides. As used herein, the term "fragment", as applied to a polypeptide, will ordinarily be at least 20 residues, more typically at least 40 residues, and preferably at least 60 residues in length. Fragments of Rps2 polypeptide can be generated by methods known to those skilled in the art. The ability of a candidate fragment to exhibit a biological activity of Rps2 can be assessed by those methods described herein. Also included in the invention are Rps2 polypeptides containing residues that are not required for biological activity of the peptide, e.g., those added by alternative mRNA splicing or alternative protein processing events.

Other embodiments are within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Ausubel, Frederick M.
Staskawicz, Brian J.
Brent, Andrew F.
Dahlbeck, Douglas
Katagiri, Fumiaki
Kunkel, Barbara N.
Mindrinos, Michael N.
Yu, Guo-Liang

(ii) TITLE OF INVENTION: RPS2 GENE AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 106

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street Suite 3100
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110-2904

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30B

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/227,360
(B) FILING DATE: 13-APR-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00786/230001

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 542-5070
(B) TELEFAX: (617) 542-8906
(C) TELEX: 100254

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2903 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGTAAAAGA AAGAGCGAGA AATCATCGAA ATGGATTTCA TCTCATCTCT TATCGTTGGC 60

TGTGCTCAGG TGTTGTGTGA ATCTATGAAT ATGGCGGAGA GAAGAGGACA TAAGACTGAT 120

CTTAGACAAG CCATCACTGA TCTTGAAACA GCCATCGGTG ACTTGAAGGC CATACGTGAT 180

GACCTGACTT TACGGATCCA ACAAGACGGT CTAGAGGGAC GAAGCTGCTC AAATCGTGCC 240

AGAGAGTGGC TTAGTGCGGT GCAAGTAACG GAGACTAAAA CAGCCCTACT TTTAGTGAGG 300

TTTAGGCGTC GGGAACAGAG GACGCGAATG AGGAGGAGAT ACCTCAGTTG TTTCGGTTGT 360

GCCGACTACA AACTGTGCAA GAAGGTTTCT GCCATATTGA AGAGCATTGG TGAGCTGAGA 420

GAACGCTCTG AAGCTATCAA AACAGATGGC GGGTCAATTC AAGTAACTTG TAGAGAGATA 480

CCCATCAAGT CCGTTGTCGG AAATACCACG ATGATGGAAC AGGTTTTGGA ATTTCTCAGT 540

GAAGAAGAAG AAAGAGGAAT CATTGGTGTT TATGGACCTG GTGGGGTTGG GAAGACAACG 600

TTAATGCAGA GCATTAACAA CGAGCTGATC ACAAAAGGAC ATCAGTATGA TGTACTGATT 660

TGGGTTCAAA TGTCCAGAGA ATTCGGCGAG TGTACAATTC AGCAAGCCGT TGGAGCACGG 720

TTGGGTTTAT CTTGGGACGA GAAGGAGACC GGCGAAAACA GAGCTTTGAA GATATACAGA 780

GCTTTGAGAC AGAAACGTTT CTTGTTGTTG CTAGATGATG TCTGGGAAGA GATAGACTTG 840

GAGAAAACTG GAGTTCCTCG ACCTGACAGG GAAAACAAAT GCAAGGTGAT GTTCACGACA 900

CGGTCTATAG CATTATGCAA CAATATGGGT GCGGAATACA AGTTGAGAGT GGAGTTTCTG 960

GAGAAGAAAC ACGCGTGGGA GCTGTTCTGT AGTAAGGTAT GGAGAAAAGA TCTTTTAGAG 1020

TCATCATCAA TTCGCCGGCT CGCGGAGATT ATAGTGAGTA AATGTGGAGG ATTGCCACTA 1080

GCGTTGATCA CTTTAGGAGG AGCCATGGCT CATAGAGAGA CAGAAGAAGA GTGGATCCAT 1140

GCTAGTGAAG TTCTGACTAG ATTTCCAGCA GAGATGAAGG GTATGAACTA TGTATTTGCC 1200

CTTTTGAAAT TCAGCTACGA CAACCTCGAG AGTGATCTGC TTCGGTCTTG TTTCTTGTAC 1260

TGCGCTTTAT TCCCAGAAGA ACATTCTATA GAGATCGAGC AGCTTGTTGA GTACTGGGTC 1320

GGCGAAGGGT TTCTCACCAG CTCCCATGGC GTTAACACCA TTTACAAGGG ATATTTTCTC 1380

ATTGGGGATC TGAAAGCGGC ATGTTTGTTG GAAACCGGAG ATGAGAAAAC ACAGGTGAAG 1440

ATGCATAATG TGGTCAGAAG CTTTGCATTG TGGATGGCAT CTGAACAGGG GACTTATAAG 1500

GAGCTGATCC TAGTTGAGCC TAGCATGGGA CATACTGAAG CTCCTAAAGC AGAAAACTGG 1560

CGACAAGCGT TGGTGATCTC ATTGTTAGAT AACAGAATCC AGACCTTGCC TGAAAAACTC 1620

ATATGCCCGA AACTGACAAC ACTGATGCTC CAACAGAACA GCTCTTTGAA GAAGATTCCA 1680

ACAGGGTTTT TCATGCATAT GCCTGTTCTC AGAGTCTTGG ACTTGTCGTT CACAAGTATC 1740

ACTGAGATTC CGTTGTCTAT CAAGTATTTG GTGGAGTTGT ATCATCTGTC TATGTCAGGA 1800

ACAAAGATAA GTGTATTGCC ACAGGAGCTT GGGAATCTTA GAAAACTGAA GCATCTGGAC 1860

CTACAAAGAA CTCAGTTTCT TCAGACGATC CCACGAGATG CCATATGTTG GCTGAGCAAG 1920

CTCGAGGTTC TGAACTTGTA CTACAGTTAC GCCGGTTGGG AACTGCAGAG CTTTGGAGAA 1980

GATGAAGCAG AAGAACTCGG ATTCGCTGAC TTGGAATACT TGGAAAACCT AACCACACTC 2040

GGTATCACTG TTCTCTCATT GGAGACCCTA AAAACTCTCT TCGAGTTCGG TGCTTTGCAT 2100

AAACATATAC AGCATCTCCA CGTTGAAGAG TGCAATGAAC TCCTCTACTT CAATCTCCCA 2160

TCACTCACTA ACCATGGCAG GAACCTGAGA AGACTTAGCA TTAAAAGTTG CCATGACTTG 2220

GAGTACCTGG TCACACCCGC AGATTTTGAA AATGATTGGC TTCCGAGTCT AGAGGTTCTG 2280

ACGTTACACA GCCTTCACAA CTTAACCAGA GTGTGGGGAA ATTCTGTAAG CCAAGATTGT 2340

CTGCGGAATA TCCGTTGCAT AAACATTTCA CACTGCAACA AGCTGAAGAA TGTCTCATGG 2400

GTTCAGAAAC TCCCAAAGCT AGAGGTGATT GAACTGTTCG ACTGCAGAGA GATAGAGGAA 2460

TTGATAAGCG AACACGAGAG TCCATCCGTC GAAGATCCAA CATTGTTCCC AAGCCTGAAG 2520

ACCTTGAGAA CTAGGGATCT GCCAGAACTA AACAGCATCC TCCCATCTCG ATTTTCATTC 2580

CAAAAAGTTG AAACATTAGT CATCACAAAT TGCCCCAGAG TTAAGAAACT GCCGTTTCAG 2640

GAGAGGAGGA CCCAGATGAA CTTGCCAACA GTTTATTGTG AGGAGAAATG GTGGAAAGCA 2700

CTGGAAAAAG ATCAACCAAA CGAAGAGCTT TGTTATTTAC CGCGCTTTGT TCCAAATTGA 2760

TATAAGAGCT AAGAGCACTC TGTACAAATA TGTCCATTCA TAAGTAGCAG GAAGCCAGGA 2820

AGGTTGTTCC AGTGAAGTCA TCAACTTTCC ACATAGCCAC AAAACTAGAG ATTATGTAAT 2880

CATAAAAACC AAACTATCCG CGA 2903

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 885 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Lys Lys Glu Arg Glu Ile Ile Glu Met Asp Phe Ile Ser Ser Leu Ile
1 5 10 15

Val Gly Cys Ala Gln Val Leu Cys Glu Ser Met Asn Met Ala Glu Arg
20 25 30

Arg Gly His Lys Thr Asp Leu Arg Gln Ala Ile Thr Asp Leu Arg Ile
35 40 45

Gln Gln Asp Gly Leu Glu Gly Arg Ser Cys Ser Asn Arg Ala Arg Glu
50 55 60

Trp Leu Ser Ala Val Gln Val Thr Glu Thr Lys Thr Ala Leu Leu Leu
65 70 75 80

Val Arg Phe Arg Arg Arg Glu Gln Arg Thr Arg Met Arg Arg Arg Tyr
85 90 95

Leu Ser Cys Phe Gly Cys Ala Asp Tyr Lys Leu Cys Lys Lys Val Ser
100 105 110

Ala Ile Leu Lys Ser Ile Gly Glu Leu Arg Glu Arg Ser Glu Ala Ile
115 120 125

Lys Thr Asp Gly Gly Ser Ile Gln Val Thr Cys Arg Glu Ile Pro Ile
130 135 140

Lys Ser Val Val Gly Asn Thr Thr Met Met Glu Gln Val Leu Glu Phe
145 150 155 160

Leu Ser Glu Glu Glu Glu Arg Gly Ile Ile Gly Val Tyr Gly Pro Gly
165 170 175

Gly Val Gly Lys Thr Thr Leu Met Gln Ser Ile Asn Asn Glu Leu Ile
180 185 190

Thr Lys Gly His Gln Tyr Asp Val Leu Ile Trp Val Gln Met Ser Arg
195 200 205

Glu Phe Gly Glu Cys Thr Ile Gln Gln Ala Val Gly Ala Arg Leu Gly
210 215 220

Leu Ser Trp Asp Glu Lys Glu Thr Gly Glu Asn Arg Ala Leu Lys Ile
225 230 235 240

Tyr Arg Ala Leu Arg Gln Lys Arg Phe Leu Leu Leu Asp Asp Val
245 250 255

Trp Glu Glu Ile Asp Leu Glu Lys Thr Gly Val Pro Arg Pro Asp Arg
260 265 270

Glu Asn Lys Cys Lys Val Met Phe Thr Thr Arg Ser Ile Ala Leu Cys
275 280 285

Asn Asn Met Gly Ala Glu Tyr Lys Leu Arg Val Glu Phe Leu Glu Lys
290 295 300

Lys His Ala Trp Glu Leu Phe Cys Ser Lys Val Trp Arg Lys Asp Leu
305 310 315 320

Leu Glu Ser Ser Ser Ile Arg Arg Leu Ala Glu Ile Ile Val Ser Lys
325 330 335

Cys Gly Gly Leu Pro Leu Ala Leu Ile Thr Leu Gly Gly Ala Met Ala
340 345 350

His Arg Glu Thr Glu Glu Glu Trp Ile His Ala Ser Glu Val Leu Thr
355 360 365

Arg Phe Pro Ala Glu Met Lys Gly Met Asn Tyr Val Phe Ala Leu Leu
370 375 380

Lys Phe Ser Tyr Asp Asn Leu Glu Ser Asp Leu Leu Arg Ser Cys Phe
385 390 395 400

Leu Tyr Cys Ala Leu Phe Pro Glu Glu His Ser Ile Glu Ile Glu Gln
405 410 415

Leu Val Glu Tyr Trp Val Gly Glu Gly Phe Leu Thr Ser Ser His Gly
420 425 430

Val Asn Thr Ile Tyr Lys Gly Tyr Phe Leu Ile Gly Asp Leu Lys Ala
435 440 445

Ala Cys Leu Leu Glu Thr Gly Asp Glu Lys Thr Gln Val Lys Met His
450 455 460

Asn Val Val Arg Ser Phe Ala Leu Trp Met Ala Ser Glu Gln Gly Thr
465 470 475 480

Tyr Lys Glu Leu Ile Leu Val Glu Pro Ser Met Gly His Thr Glu Ala
485 490 495

Pro Lys Ala Glu Asn Trp Arg Gln Ala Leu Val Ile Ser Leu Leu Asp
500 505 510

Asn Arg Ile Gln Thr Leu Pro Glu Lys Leu Ile Cys Pro Lys Leu Thr
515 520 525

Thr Leu Met Leu Gln Gln Asn Ser Ser Leu Lys Lys Ile Pro Thr Gly
530 535 540

Phe Phe Met His Met Pro Val Leu Arg Val Leu Asp Leu Ser Phe Thr
545 550 555 560

Ser Ile Thr Glu Ile Pro Leu Ser Ile Lys Tyr Leu Val Glu Leu Tyr
565 570 575

His Leu Ser Met Ser Gly Thr Lys Ile Ser Val Leu Pro Gln Glu Leu
580 585 590

Gly Asn Leu Arg Lys Leu Lys His Leu Asp Leu Gln Arg Thr Gln Phe
595 600 605

Leu Gln Thr Ile Pro Arg Asp Ala Ile Cys Trp Leu Ser Lys Leu Glu
610 615 620

Val Leu Asn Leu Tyr Tyr Ser Tyr Ala Gly Trp Glu Leu Gln Ser Phe
625 630 635 640

Gly Glu Asp Glu Ala Glu Glu Leu Gly Phe Ala Asp Leu Glu Tyr Leu
645 650 655

Glu Asn Leu Thr Thr Leu Gly Ile Thr Val Leu Ser Leu Glu Thr Leu
660 665 670

Lys Thr Leu Phe Glu Phe Gly Ala Leu His Lys His Ile Gln His Leu
675 680 685

His Val Glu Glu Cys Asn Glu Leu Leu Tyr Phe Asn Leu Pro Ser Leu
690 695 700

Thr Asn His Gly Arg Asn Leu Arg Arg Leu Ser Ile Lys Ser Cys His
705 710 715 720

Asp Leu Glu Tyr Leu Val Thr Pro Ala Asp Phe Glu Asn Asp Trp Leu
725 730 735

Pro Ser Leu Glu Val Leu Thr Leu His Ser Leu His Asn Leu Arg Cys
740 745 750

Ile Asn Ile Ser His Cys Asn Lys Leu Lys Asn Val Ser Trp Val Gln
755 760 765

Lys Leu Pro Lys Leu Glu Val Ile Glu Leu Phe Asp Cys Arg Glu Ile
770 775 780

```
Glu Glu Leu Ile Ser Glu His Glu Ser Pro Ser Val Glu Asp Pro Thr
785                 790                 795                 800

Leu Phe Pro Ser Leu Lys Thr Leu Arg Thr Arg Asp Leu Pro Glu Leu
                805                 810                 815

Asn Ser Ile Leu Pro Ser Arg Phe Ser Phe Gln Lys Val Glu Thr Leu
            820                 825                 830

Val Ile Thr Asn Cys Pro Arg Val Lys Lys Leu Pro Phe Gln Glu Arg
        835                 840                 845

Arg Thr Gln Met Asn Leu Pro Thr Val Tyr Cys Glu Glu Lys Trp Trp
    850                 855                 860

Lys Ala Leu Glu Lys Asp Gln Pro Asn Glu Glu Leu Cys Tyr Leu Pro
865                 870                 875                 880

Arg Phe Val Pro Asn
                885
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Glu His Ser Val Gln Ile Cys Pro Phe Ile Ser Ser Arg Lys Pro Gly
1               5                   10                  15

Arg Leu Phe Gln
            20
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ser His Gln Leu Ser Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Arg Leu Cys Asn His Lys Asn Gln Thr Ile Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Lys Arg Lys Ser Glu Lys Ser Ser Lys Trp Ile Ser Ser His Leu
1 5 10 15

Leu Ser Leu Ala Val Leu Arg Cys Cys Val Asn Leu
20 25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ile Trp Arg Arg Glu Glu Asp Ile Arg Leu Ile Leu Asp Lys Pro Ser
1 5 10 15

Leu Ile Leu Lys Gln Pro Ser Val Thr
20 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Arg Pro Tyr Val Met Thr
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Leu Tyr Gly Ser Asn Lys Thr Val
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Asp Glu Ala Ala Gln Ile Val Pro Glu Ser Gly Leu Val Arg Cys
1 5 10 15

Lys

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Arg Arg Leu Lys Gln Pro Tyr Phe
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gly Leu Gly Val Gly Asn Arg Gly Arg Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gly Gly Asp Thr Ser Val Val Ser Val Val Pro Thr Thr Asn Cys Ala
1 5 10 15

Arg Arg Phe Leu Pro Tyr
20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Arg Ala Leu Val Ser
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Asn Ala Leu Lys Leu Ser Lys Gln Met Ala Gly Gln Phe Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Leu Val Glu Arg Tyr Pro Ser Ser Pro Leu Ser Glu Ile Pro Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Trp Asn Arg Phe Trp Asn Phe Ser Val Lys Lys Lys Glu Glu Ser
1 5 10 15

Leu Val Phe Met Asp Leu Val Gly Leu Gly Arg Gln Arg
20 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Cys Arg Ala Leu Thr Thr Ser
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ser Gln Lys Asp Ile Ser Met Met Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Phe Gly Phe Lys Cys Pro Glu Asn Ser Ala Ser Val Gln Phe Ser Lys
1 5 10 15

Pro Leu Glu His Gly Trp Val Tyr Leu Gly Thr Arg Arg Arg Pro Ala
20 25 30

Lys Thr Glu Leu
35

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Arg Tyr Thr Glu Leu
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Asp Arg Asn Val Ser Cys Cys Cys
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Met Ser Gly Lys Arg
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Thr Trp Arg Lys Leu Glu Phe Leu Asp Leu Thr Gly Lys Thr Asn Ala
1 5 10 15

Arg

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Cys Ser Arg His Gly Leu
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

His Tyr Ala Thr Ile Trp Val Arg Asn Thr Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Glu Trp Ser Phe Trp Arg Arg Asn Thr Arg Gly Ser Cys Ser Val Val
1 5 10 15

Arg Tyr Gly Glu Lys Ile Phe
20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ser His His Gln Phe Ala Gly Ser Arg Arg Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Val Asn Val Glu Asp Cys His
1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Glu Glu Pro Trp Leu Ile Glu Arg Gln Lys Lys Ser Gly Ser Met Leu
1 5 10 15

Val Lys Phe

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Leu Asp Phe Gln Gln Arg
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Thr Met Tyr Leu Pro Phe
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Asn Ser Ala Thr Thr Thr Ser Arg Val Ile Cys Phe Gly Leu Val Ser
1 5 10 15

Cys Thr Ala Leu Tyr Ser Gln Lys Asn Ile Leu
20 25

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Lys Arg His Val Cys Trp Lys Pro Glu Met Arg Lys His Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Arg Ser Ser Ser Leu Leu Ser Thr Gly Ser Ala Lys Gly Phe Ser Pro
1 5 10 15
Ala Pro Met Ala Leu Thr Pro Phe Thr Arg Asp Ile Phe Ser Leu Gly
20 25 30

Ile

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Arg Cys Ile Met Trp Ser Glu Ala Leu His Cys Gly Trp His Leu Asn
1 5 10 15

Arg Gly Leu Ile Arg Ser
20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Leu Ser Leu Ala Trp Asp Ile Leu Lys Leu Leu Lys Gln Lys Thr Gly
1 5 10 15

Asp Lys Arg Trp
20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Ile Thr Glu Ser Arg Pro Cys Leu Lys Asn Ser Tyr Ala Arg Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Cys Ser Asn Arg Thr Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Arg Arg Phe Gln Gln Gly Phe Ser Cys Ile Cys Leu Phe Ser Glu Ser
1 5 10 15

Trp Thr Cys Arg Ser Gln Val Ser Leu Arg Phe Arg Cys Leu Ser Ser
20 25 30

Ile Trp Trp Ser Cys Ile Ile Cys Leu Cys Gln Glu Gln Arg
35 40 45

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Val Tyr Cys His Arg Ser Leu Gly Ile Leu Glu Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Ser Ile Trp Thr Tyr Lys Glu Leu Ser Phe Phe Arg Arg Ser His Glu
1 5 10 15

Met Pro Tyr Val Gly
20

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Ala Ser Ser Arg Phe
1 5

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Thr Cys Thr Thr Val Thr Pro Val Gly Asn Cys Arg Ala Leu Glu Lys
1 5 10 15

Met Lys Gln Lys Asn Ser Asp Ser Leu Thr Trp Asn Thr Trp Lys Thr
20 25 30

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Pro His Ser Val Ser Leu Phe Ser His Trp Arg Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Lys Leu Ser Ser Ser Ser Val Leu Cys Ile Asn Ile Tyr Ser Ile Ser
1 5 10 15

Thr Leu Lys Ser Ala Met Asn Ser Ser Thr Ser Ile Ser His His Ser
20 25 30

Leu Thr Met Ala Gly Thr
35

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Glu Asp Leu Ala Leu Lys Val Ala Met Thr Trp Ser Thr Trp Ser His
1 5 10 15

Pro Gln Ile Leu Lys Met Ile Gly Phe Arg Val
20 25

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Arg Tyr Thr Ala Phe Thr Thr
1 5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Pro Glu Cys Gly Glu Ile Leu
1 5

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Phe Arg Asn Ser Gln Ser
1 5

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Ala Lys Ile Val Cys Gly Ile Ser Val Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Thr Phe His Thr Ala Thr Ser
1 5

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Leu Asn Cys Ser Thr Ala Glu Arg
1 5

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Ala Asn Thr Arg Val His Pro Ser Lys Ile Gln His Cys Ser Gln Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Glu Leu Gly Ile Cys Gln Asn
1 5

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Thr Ala Ser Ser His Leu Asp Phe His Ser Lys Lys Leu Lys His
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Ser Ser Gln Ile Ala Pro Glu Leu Arg Asn Cys Arg Phe Arg Arg Gly
1 5 10 15

Gly Pro Arg

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Thr Cys Gln Gln Phe Ile Val Arg Arg Asn Gly Gly Lys His Trp Lys
1 5 10 15

Lys Ile Asn Gln Thr Lys Ser Phe Val Ile Tyr Arg Ala Leu Phe Gln
20 25 30

Ile Asp Ile Arg Ala Lys Ser Thr Leu Tyr Lys Tyr Val His Ser
35 40 45

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Val Ala Gly Ser Gln Glu Gly Cys Ser Ser Glu Val Ile Asn Phe Pro
1 5 10 15

His Ser His Lys Thr Arg Asp Tyr Val Ile Ile Lys Thr Lys Leu Ser
20 25 30

Ala

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Val Lys Glu Arg Ala Arg Asn His Arg Asn Gly Phe His Leu Ile Ser
1 5 10 15

Tyr Arg Trp Leu Cys Ser Gly Val Val
20 25

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Ile Tyr Glu Tyr Gly Gly Glu Lys Arg Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Leu Glu Gly His Thr
1 5

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Pro Asp Phe Thr Asp Pro Thr Arg Arg Ser Arg Gly Thr Lys Leu Leu
1 5 10 15

Lys Ser Cys Gln Arg Val Ala
20

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Cys Gly Ala Ser Asn Gly Asp
1 5

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Asn Ser Pro Thr Phe Ser Glu Val
1 5

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Ala Ser Gly Thr Glu Asp Ala Asn Glu Glu Glu Ile Pro Gln Leu Phe
1 5 10 15

Arg Leu Cys Arg Leu Gln Thr Val Gln Glu Gly Phe Cys His Ile Glu
20 25 30

Glu His Trp
35

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Ala Glu Arg Thr Leu
1 5

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Ser Tyr Gln Asn Arg Trp Arg Val Asn Ser Ser Asn Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Arg Asp Thr His Gln Val Arg Cys Arg Lys Tyr His Asp Asp Gly Thr
1 5 10 15

Gly Phe Gly Ile Ser Gln
20

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Arg Arg Arg Lys Arg Asn His Trp Cys Leu Trp Thr Trp Trp Gly Trp
1 5 10 15

Glu Asp Asn Val Asn Ala Glu His
20

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Gln Arg Ala Asp His Lys Arg Thr Ser Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Cys Thr Asp Leu Gly Ser Asn Val Gln Arg Ile Arg Arg Val Tyr Asn
1 5 10 15

Ser Ala Ser Arg Trp Ser Thr Val Gly Phe Ile Leu Gly Arg Glu Gly
20 25 30

Asp Arg Arg Lys Gln Ser Phe Glu Asp Ile Gln Ser Phe Glu Thr Glu
35 40 45

Thr Phe Leu Val Val Ala Arg
50 55

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Cys Leu Gly Arg Asp Arg Leu Gly Glu Asn Trp Ser Ser Ser Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Arg Asp Arg Arg Arg Val Asp Pro Cys
1 5

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Gln Gly Lys Gln Met Gln Gly Asp Val His Asp Thr Val Tyr Ser Ile
1 5 10 15

Met Gln Gln Tyr Gly Cys Gly Ile Gln Val Glu Ser Gly Val Ser Gly
20 25 30

Glu Glu Thr Arg Val Gly Ala Val Leu
35 40

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Gly Met Glu Lys Arg Ser Phe Arg Val Ile Ile Asn Ser Pro Ala Arg
1 5 10 15

Gly Asp Tyr Ser Glu
20

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Met Trp Arg Ile Ala Thr Ser Val Asp His Phe Arg Arg Ser His Gly
1 5 10 15

Ser

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Ile Ser Ser Arg Asp Glu Gly Tyr Glu Leu Cys Ile Cys Pro Phe Glu
1 5 10 15

Ile Gln Leu Arg Gln Pro Arg Glu
20

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Ser Ala Ser Val Leu Phe Leu Val Leu Arg Phe Ile Pro Arg Arg Thr
1 5 10 15

Phe Tyr Arg Asp Arg Ala Ala Cys
20

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Val Leu Gly Arg Arg Arg Val Ser His Gln Leu Pro Trp Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

```
His His Leu Gln Gly Ile Phe Ser His Trp Gly Ser Glu Ser Gly Met
1               5                  10                  15

Phe Val Gly Asn Arg Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

```
Glu Asn Thr Gly Glu Asp Ala
1               5
```

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

```
Lys Thr His Met Pro Glu Thr Asp Asn Thr Asp Ala Pro Thr Glu Gly
1               5                  10                  15

Leu Phe Glu Glu Asp Ser Asn Arg Val Phe His Ala Tyr Ala Cys Ser
            20                  25                  30

Gln Ser Leu Gly Leu Val Val His Lys Tyr His
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```
Cys Gly Gln Lys Leu Cys Ile Val Asp Gly Ile
1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Gly Ala Asp Pro Ser
1 5

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Ser Arg Lys Leu Ala Thr Ser Val Gly Asp Leu Ile Val Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Gln Asn Pro Asp Leu Ala
1 5

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Asp Ser Val Val Tyr Gln Val Phe Gly Gly Val Val Ser Ser Val Tyr
1 5 10 15

Val Arg Asn Lys Asp Lys Cys Ile Ala Thr Gly Ala Trp Glu Ser
20 25 30

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Lys Thr Glu Ala Ser Gly Pro Thr Lys Asn Ser Val Ser Ser Asp Asp
1 5 10 15

Pro Thr Arg Cys His Met Leu Ala Glu Gln Ala Arg Gly Ser Glu Leu
20 25 30

Val Leu Gln Leu Arg Arg Leu Gly Thr Ala Glu Leu Trp Arg Arg
35 40 45

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Ser Arg Arg Thr Arg Ile Arg
1 5

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Leu Gly Ile Leu Gly Lys Pro Asn His Thr Arg Tyr His Cys Ser Leu
1 5 10 15

Ile Gly Asp Pro Lys Asn Ser Leu Arg Val Arg Cys Phe Ala
20 25 30

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Thr Tyr Thr Ala Ser Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Thr Pro Leu Leu Gln Ser Pro Ile Thr His
1 5 10

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Pro Trp Gln Glu Pro Glu Lys Thr
1 5

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Leu Gly Val Pro Gly His Thr Arg Arg Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 58 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

Leu Ala Ser Glu Ser Arg Gly Ser Asp Val Thr Gln Pro Ser Gln Leu

1 5 10 15

Asn Gln Ser Val Gly Lys Phe Cys Lys Pro Arg Leu Ser Ala Glu Tyr
20 25 30

Pro Leu His Lys His Phe Thr Leu Gln Gln Ala Glu Glu Cys Leu Met
35 40 45

Gly Ser Glu Thr Pro Lys Ala Arg Gly Asp
50 55

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Thr Val Arg Leu Gln Arg Asp Arg Gly Ile Asp Lys Arg Thr Arg Glu
1 5 10 15

Ser Ile Arg Arg Arg Ser Asn Ile Val Pro Lys Pro Glu Asp Leu Glu
20 25 30

Asn

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

Gly Ser Ala Arg Thr Lys Gln His Pro Pro Ile Ser Ile Phe Ile Pro
1 5 10 15

Lys Ser

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

Asn Ile Ser His His Lys Leu Pro Gln Ser

1 5 10

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

Glu Thr Ala Val Ser Gly Glu Glu Asp Pro Asp Glu Leu Ala Asn Ser
1 5 10 15

Leu Leu

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GGTAGTGAGT AGAGAATAAC 20

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

Glu Leu Arg Ala Leu Cys Thr Asn Met Ser Ile His Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

Gln Glu Ala Arg Lys Val Val Pro Val Lys Ser Ser Thr Phe His Ile
1 5 10 15

Ala Thr Lys Leu Glu Ile Met
20

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Lys Pro Asn Tyr Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

ATCGATTGAT CTCTGGCTCA GTGCGAGTAG TCCATTTGAG AGCAGTCGTA GCCCCGCGTG 60

GCGCATCATG GAGCTATTTG GAATTTTCGC AGGGTTATCG ATTCGTAGTG GGAACCCATT 120

CATTGTTTGG AACCACCAAC GGACGACTTA ACAAGCTCCC CGAGGTGCAT GATGAAAATT 180

GCTCCAGTTG CCATAAATCA CAGCCCGCTC AGCAGGGAGG TCCCGTCACA CGCGGCACCC 240

ACTCAGGCAA AGCAAACCAA CCTTCAATCT GAAGCTGGCG ATTTAGATGC AAGAAAAAGT 300

AGCGCTTCAA GCCCGGAAAC CCGCGCATTA CTCGCTACTA AGACAGTACT CGGGAGACAC 360

AAGATAGAGG TTCCGGCCTT TGGAGGGTGG TTCAAAAAGA AATCATCTAA GCACGAGACG 420

GGCGGTTCAA GTGCCAACGC AGATAGTTCG AGCGTGGCTT CCGATTCCAC CGAAAAACCT 480

TTGTTCCGTC TCACGCACGT TCCTTACGTA TCCCAAGGTA ATGAGCGAAT GGGATGTTGG 540

TATGCCTGCG CAAGAATGGT TGGCCATTCT GTCGAAGCTG GGCCTCGCCT AGGGCTGCCG 600

GAGCTCTATG AGGGAAGGGA GGCGCCAGCT GGGCTACAAG ATTTTTCAGA TGTAGAAAGG 660

TTTATTCACA ATGAAGGATT AACTCGGGTA GACCTTCCAG ACAATGAGAG ATTTACACAC 720

GAAGAGTTGG GTGCACTGTT GTATAAGCAC GGGCCGATTA TATTTGGGTG GAAAACTCCG 780

AATGACAGCT GGCACATGTC GGTCCTCACT GGTGTCGATA AAGAGACGTC GTCCATTACT 840

TTTCACGATC CCCGACAGGG GCCGGACCTA GCAATGCCGC TCGATTACTT TAATCAGCGA 900

TTGGCATGGC AGGTTCCACA CGCAATGCTC TACCGCTAAG TAGCAGGGTA TCTTCACGTG 960

GCGGCATCAT GACAAGCCCA TGATGCCGCC AGCAGCTACC TGAATGCCGT CTGGCTTTTT 1020

GGTCCCTATT GTCGTATCCG GAAGATGACG TCAAAGAATC TCGGCAAGAG CTTTCTTGCT 1080

CGACTCCTCA GCTTCCGGAT CGATCAGGTC GCTTGCCAGA GCGCGCTTGT CCATGAGCAT 1140

CTGCCACAGC TGCTGGTCGA TGGTGTCCTC AGCTAAAGGG ATTTTGACGA CAACCATGCG 1200

CAACTGCCCG TTGCGATACG CTCGATCCTG AAGCCCCGGT GTCCATGGCA GCCCCAAGAA 1260

AAAGACATAG TTCGCCGCTG TGAGGTTGTA GCCTGTGCCG GCGGCCGACC TGGTCCCGAT 1320

AAACACCCTG CAGTCCGGAT CCTGCTGGAA AGCATCAATC GCCTTCTGCC GCTTCTTGGG 1380

CGAGTCACTG CCCACCAACG TCACGCACCC GACGCCAAGC TTGAGGCAGT GCTCCCGCAA 1440

CGTGGCCACG GATTCCTGAT ACTCGCAGAA GAGGATCACC TTGTCGTCGA C 1491

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 255 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

Met Lys Ile Ala Pro Val Ala Ile Asn His Ser Pro Leu Ser Arg Glu
1 5 10 15

Val Pro Ser His Ala Ala Pro Thr Gln Ala Lys Gln Thr Asn Leu Gln
20 25 30

Ser Glu Ala Gly Asp Leu Asp Ala Arg Lys Ser Ser Ala Ser Ser Pro
35 40 45

Glu Thr Arg Ala Leu Leu Ala Thr Lys Thr Val Leu Gly Arg His Lys
50 55 60

Ile Glu Val Pro Ala Phe Gly Gly Trp Phe Lys Lys Lys Ser Ser Lys
65 70 75 80

His Glu Thr Gly Gly Ser Ser Ala Asn Ala Asp Ser Ser Ser Val Ala
85 90 95

Ser Asp Ser Thr Glu Lys Pro Leu Phe Arg Leu Thr His Val Pro Tyr
100 105 110

Val Ser Gln Gly Asn Glu Arg Met Gly Cys Trp Tyr Ala Cys Ala Arg
115 120 125

Met Val Gly His Ser Val Glu Ala Gly Pro Arg Leu Gly Leu Pro Glu
130 135 140

Leu Tyr Glu Gly Arg Glu Ala Pro Ala Gly Leu Gln Asp Phe Ser Asp
145 150 155 160

Val Glu Arg Phe Ile His Asn Glu Gly Leu Thr Arg Val Asp Leu Pro
165 170 175

Asp Asn Glu Arg Phe Thr His Glu Glu Leu Gly Ala Leu Leu Tyr Lys
180 185 190

His Gly Pro Ile Ile Phe Gly Trp Lys Thr Pro Asn Asp Ser Trp His
195 200 205

Met Ser Val Leu Thr Gly Val Asp Lys Glu Thr Ser Ser Ile Thr Phe
210 215 220

His Asp Pro Arg Gln Gly Pro Asp Leu Ala Met Pro Leu Asp Tyr Phe
225 230 235 240

Asn Gln Arg Leu Ala Trp Gln Val Pro His Ala Met Leu Tyr Arg
245 250 255

What is claimed is:

Claims

1. Substantially pure DNA encoding an *Rps* polypeptide.

2. The DNA of claim 1, wherein said DNA contains the *RPS2* gene [SEQ. ID. NO:1].

3. The DNA of claim 1, wherein said DNA is genomic DNA.

4. The DNA of claim 1, wherein said DNA is cDNA.

5. The DNA of claim 1, wherein said DNA is of a plant of the genus *Arabidopsis*.

6. Substantially pure DNA having the sequence [SEQ. ID NO:1] of Fig. 2, or degenerate variants thereof, and encoding the amino acid sequence [SEQ. ID NOS:2-5] of open reading frame "a" of Fig. 2.

7. Substantially pure DNA having about 50% or greater sequence identity to the DNA sequence [SEQ. ID. NO:1] of Fig. 2.

8. The DNA of claim 1 or 2, wherein said DNA is operably linked to regulatory sequences for expression of said polypeptide; and

wherein said regulatory sequences comprise a promoter.

9. The DNA of claim 8, wherein said promoter is a constitutive promoter.

10. The DNA of claim 8, wherein said promoter is inducible by one or more external agents.

11. The DNA of claim 8, wherein said promoter is cell-type specific.

12. A cell which contains the DNA of claim 1.

13. The cell of claim 12, said cell being a plant cell.

14. The plant cell of claim 13, said plant cell being resistant to disease caused by a plant pathogen carrying an avirulence gene generating a signal recognized by an Rps polypeptide.

15. The plant cell of claim 14, said plant pathogen carrying an *avrRpt2* gene.

16. The plant cell of claim 14, said plant cell being from the group of plants comprising *Arabidopsis*, tomato, soybean, bean, maize, wheat, and rice.

17. The plant cell of claim 14, said plant pathogen being *Pseudomonas syringae*.

18. The plant cell of claim 13, wherein said plant cell further contains an *avrRpt2* gene operably linked to regulatory sequences; and

wherein said regulatory sequences comprise a promoter.

19. The plant cell of claim 18, wherein said promoter is a constitutive promoter.

20. The plant cell of claim 18, wherein said promoter is inducible by one or more external agents.

21. The plant cell of claim 18, wherein said promoter is cell-type specific.

22. A transgenic plant which contains the DNA of claim 1 integrated into the genome of said plant, wherein said DNA is expressed in said transgenic plant.

23. A transgenic plant which contains the DNA of claim 8 integrated into the genome of said plant, wherein said DNA is expressed in said transgenic plant.

24. A transgenic plant generated from the plant cell of claim 18 wherein said DNA and said *avrRpt2* gene are expressed in said transgenic plant.

25. A seed from a transgenic plant of claim 22.

26. A seed from a transgenic plant of claim 23.

27. A seed from a transgenic plant of claim 24.

28. A cell from a transgenic plant of claim 22.

29. A cell from a transgenic plant of claim 23.

30. A method of providing resistance to a plant pathogen in a plant, said method comprising:

producing a transgenic plant cell comprising the DNA of claim 1 integrated into the genome of said transgenic plant cell and positioned for expression in said plant cell; and

growing a transgenic plant from said plant cell wherein said DNA is expressed in said transgenic plant.

31. A method of detecting a resistance gene in a plant cell, said method comprising:

contacting the DNA of claim 1 or a portion thereof greater than about 18 nucleic acids in length with a preparation of genomic DNA from said plant cell under hybridization conditions providing detection of DNA sequences having about 50% or greater sequence identity to the sequence [SEQ. ID. NO:1] of Fig.2.

32. A method of producing an Rps2 polypeptide comprising:

providing a cell transformed with DNA encoding an Rps2 polypeptide positioned for expression in said cell; culturing said transformed cell under conditions for expressing said DNA; and

isolating said Rps2 polypeptide.

33. A method of providing, in a transgenic plant, resistance to a plant pathogen, said method comprising:

producing a transgenic plant cell comprising the DNA of claim 8 integrated into the genome of said transgenic plant cell and positioned for expression in said plant cell; and

growing said transgenic plant from said plant cell wherein said DNA is expressed in said transgenic plant.

34. A method of providing, in a transgenic plant, resistance to a plant pathogen, said method comprising:

growing said transgenic plant from the plant cell of claim 18 wherein said DNA and said *avrRpt2* gene are expressed in said transgenic plant.

35. A method of isolating a disease resistance gene or portion thereof in plants having sequence identity to *RPS2*, [SEQ. ID NO:1] said method comprising:

amplifying by PCR said disease resistance gene or portion thereof using oligonucleotide primers wherein said primers

(a) are each greater than 13 nucleotides in length;

(b) each have regions of complementarity to opposite DNA strands in a region of the nucleotide sequence [SEQ. ID NO:1] of Fig. 2; and

(c) optionally contain sequences capable of producing restriction enzyme cut sites in the amplified product; and

isolating said disease resistance gene or portion thereof.

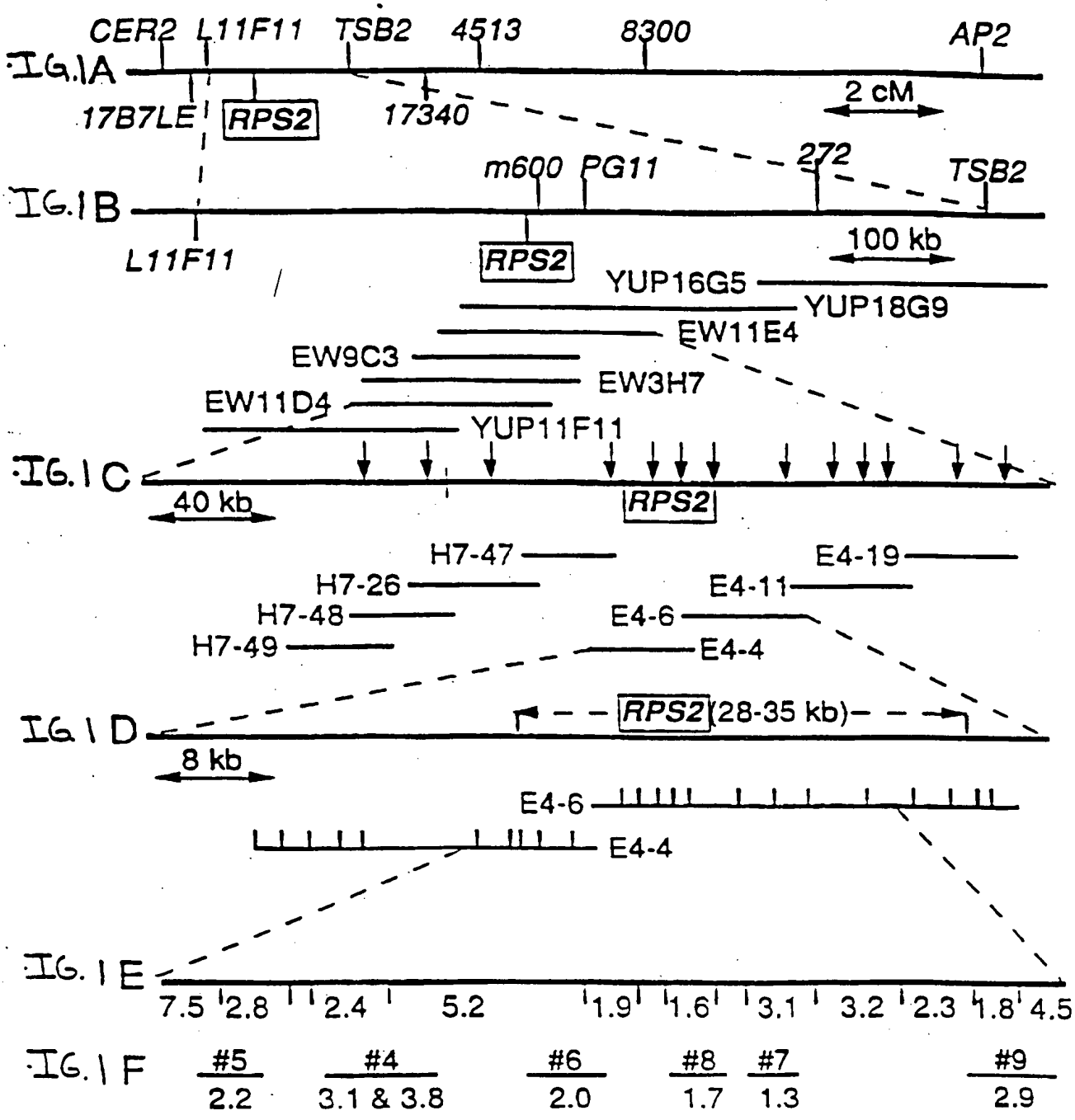
36. A substantially pure Rps2 polypeptide.

37. The polypeptide of claim 32, comprising an amino acid sequence substantially identical to an amino acid sequence [SEQ. ID NOS:2-5] shown in Fig. 2.

38. A vector comprising the DNA of claim 1, said vector being capable of directing expression of the peptide encoded by said DNA in a vector-containing cell.

39. A vector comprising the DNA of the *avrRpt2* [SEQ. ID NO:105] gene operably linked to regulatory sequences wherein said regulatory sequences comprise a promoter.

40. A vector comprising the DNA of claim 1 and the DNA of the *avrRpt2* gene [SEQ. ID NO:105] operably linked to regulatory sequences wherein said regulatory sequences comprise a promoter.


CER2
L11F11
TSB2
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8300
AP2
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RPS2
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PG11
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TSB2
IG.1B
L11F11
RPS2
100 kb
YUP16G5
YUP18G9
EW11E4
EW9C3
EW3H7
EW11D4
YUP11F11
IG.1C
40 kb
RPS2
H7-47
E4-19
H7-26
E4-11
H7-48
E4-6
H7-49
E4-4
IG.1D
RPS2 (28-35 kb)
8 kb
E4-6
E4-4
IG.1E
7.5
2.8
2.4
5.2
1.9
1.6
3.1
3.2
2.3
1.8
4.5
IG.1F
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#4
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#6
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#8
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#7
1.3
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2.9

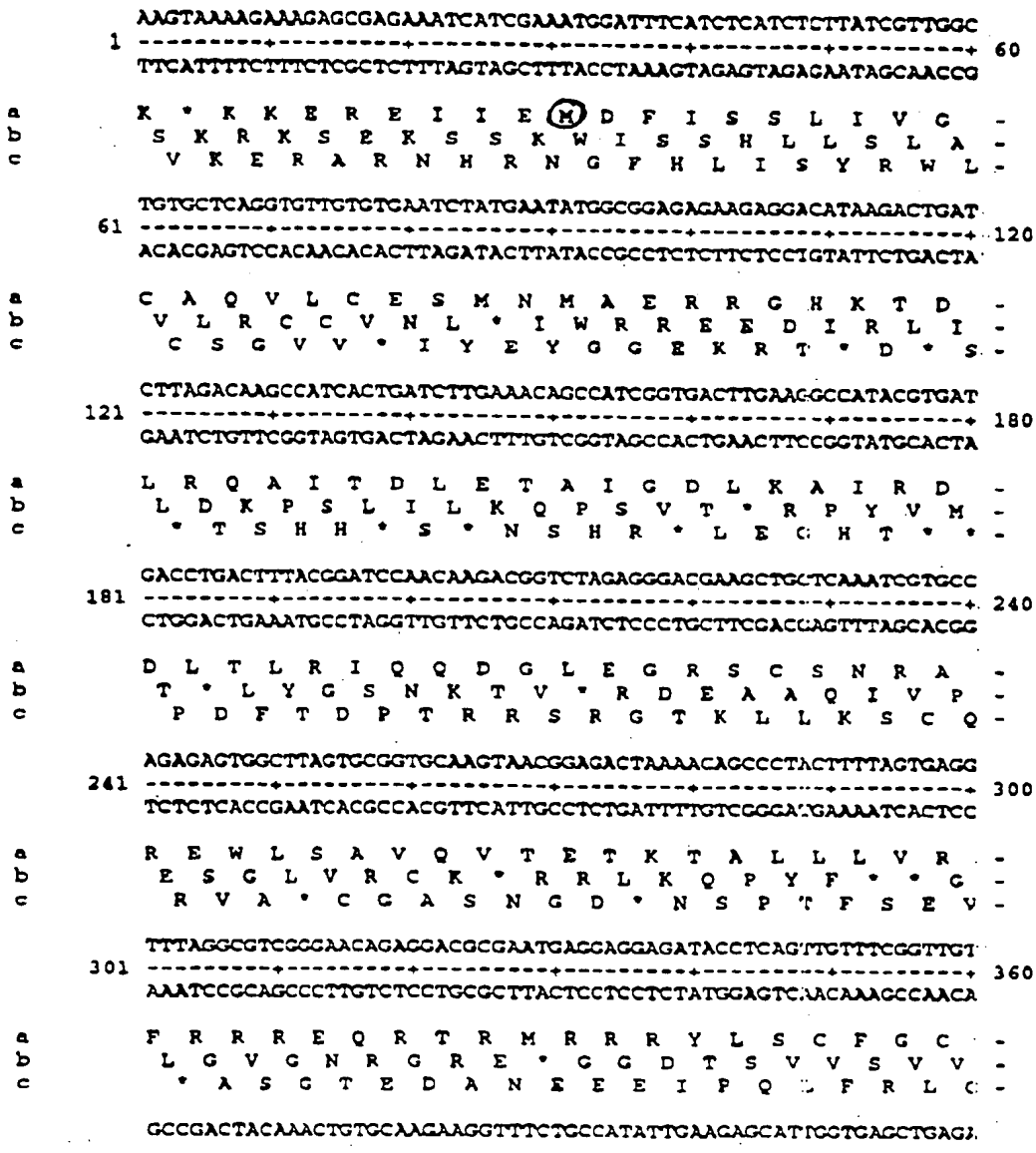

FIGURE 2

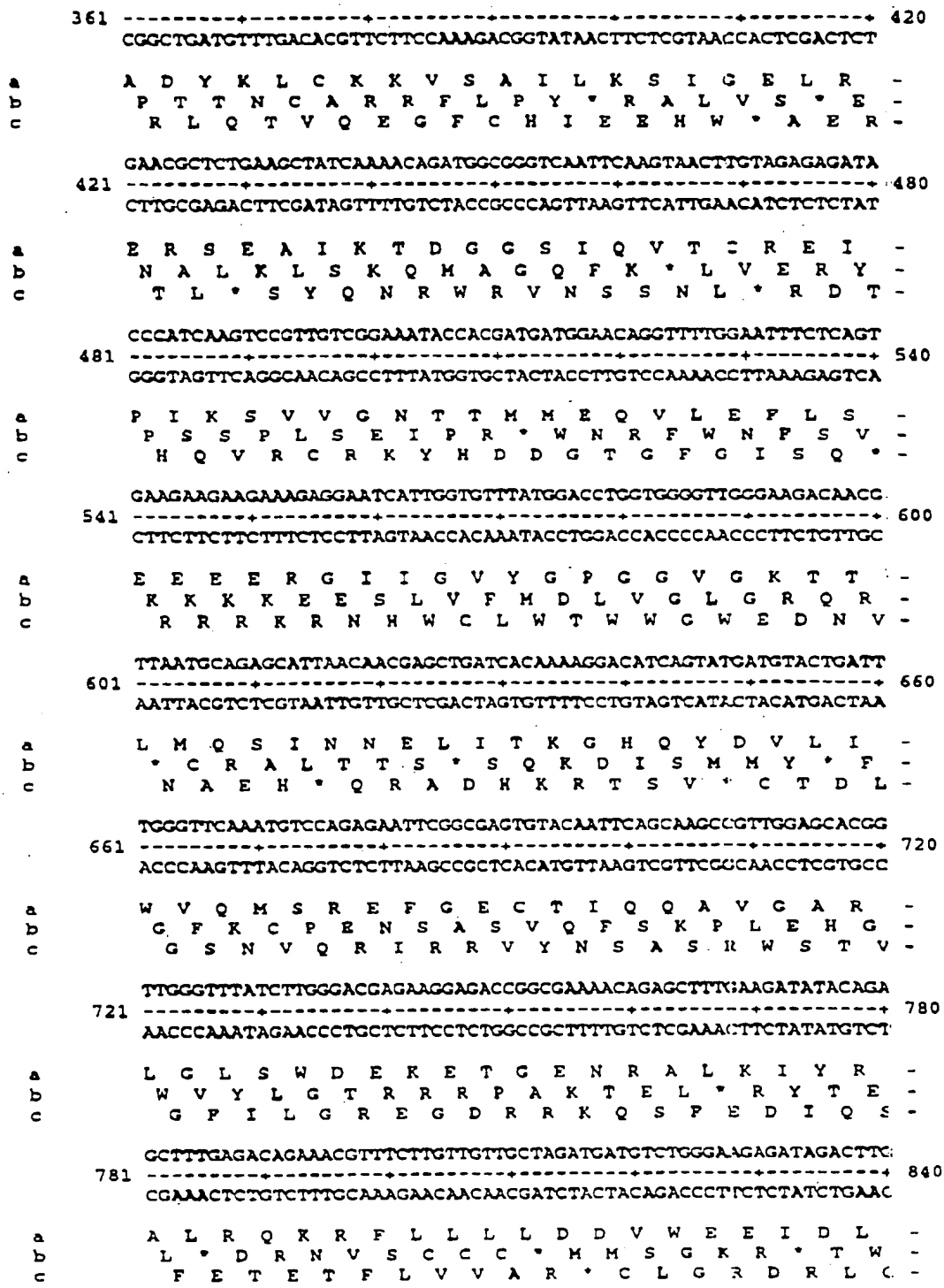

FIG. 2 CONTINUED

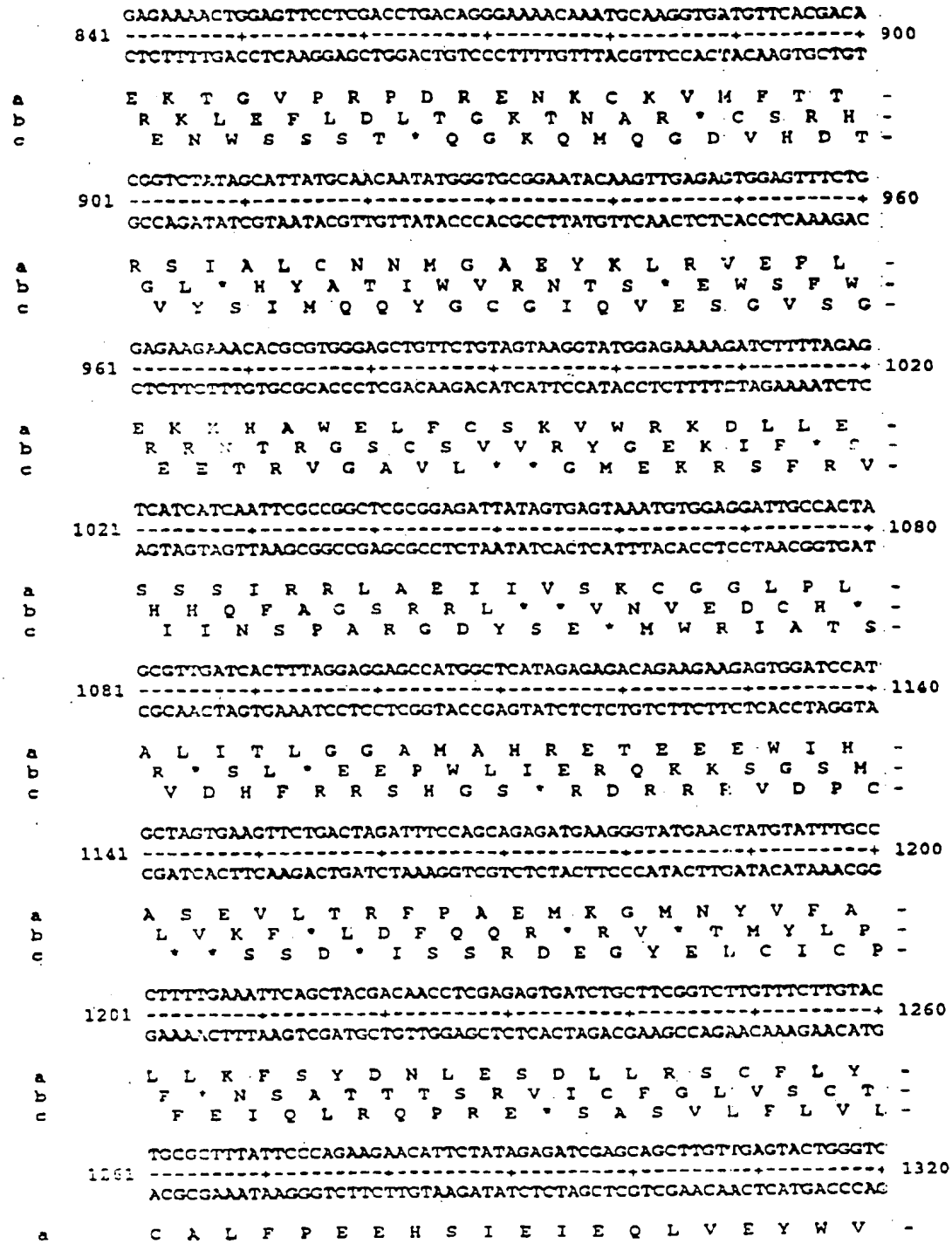

FIG. 2 CONTINUED

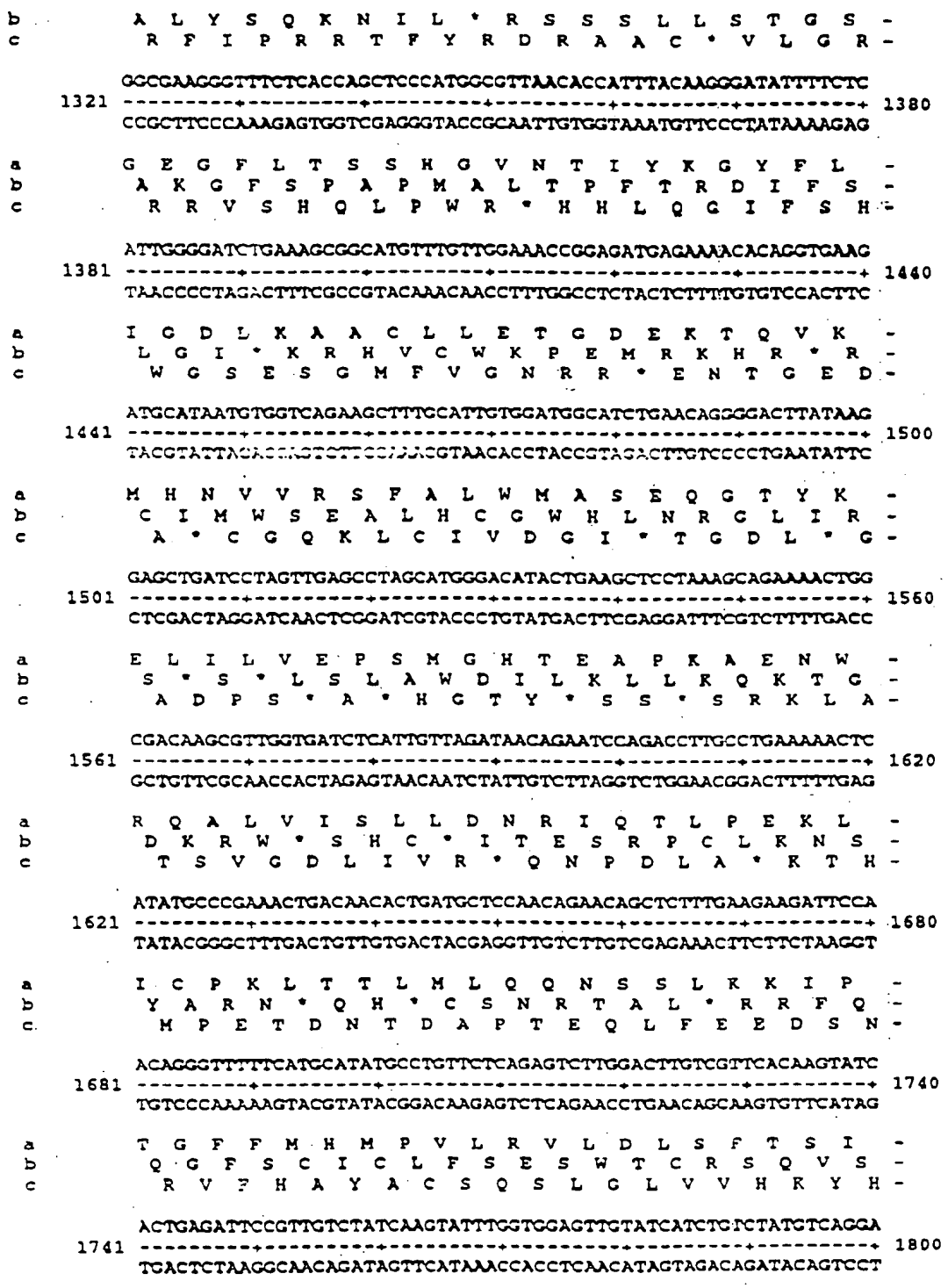

FIG. 2 CONTINUED

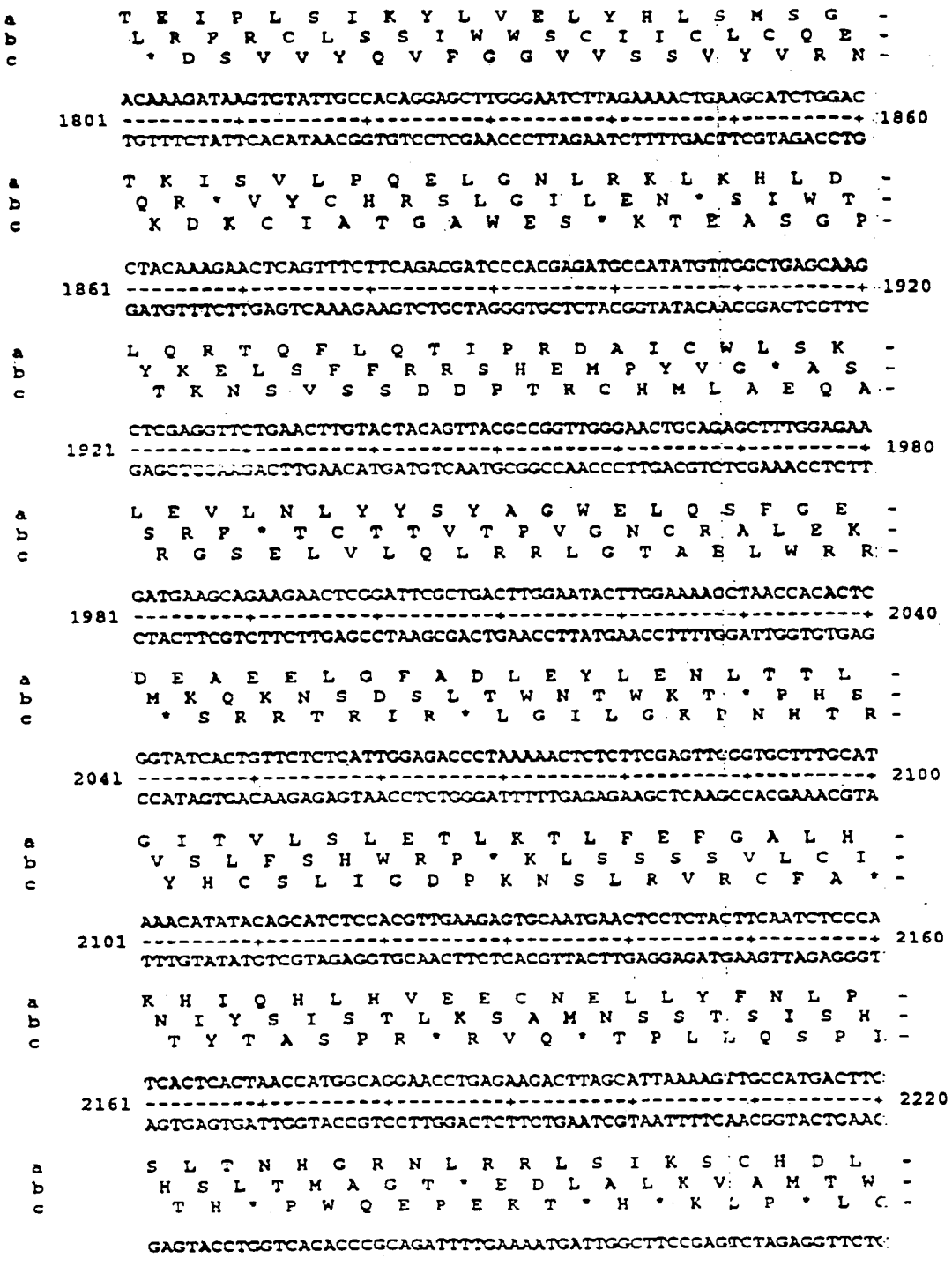

FIG. 2 CONTINUED

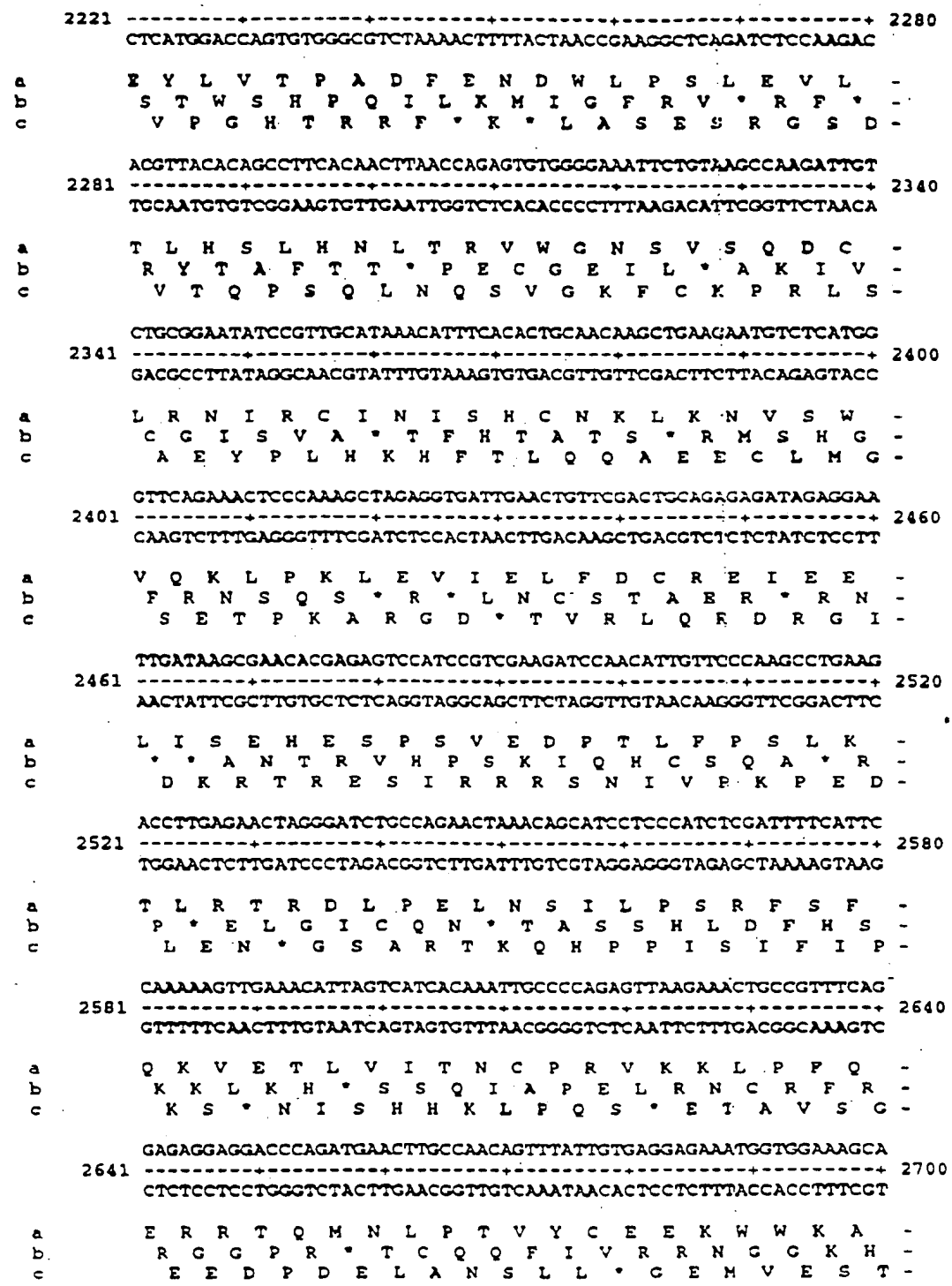

FIG. 2 CONTINUED

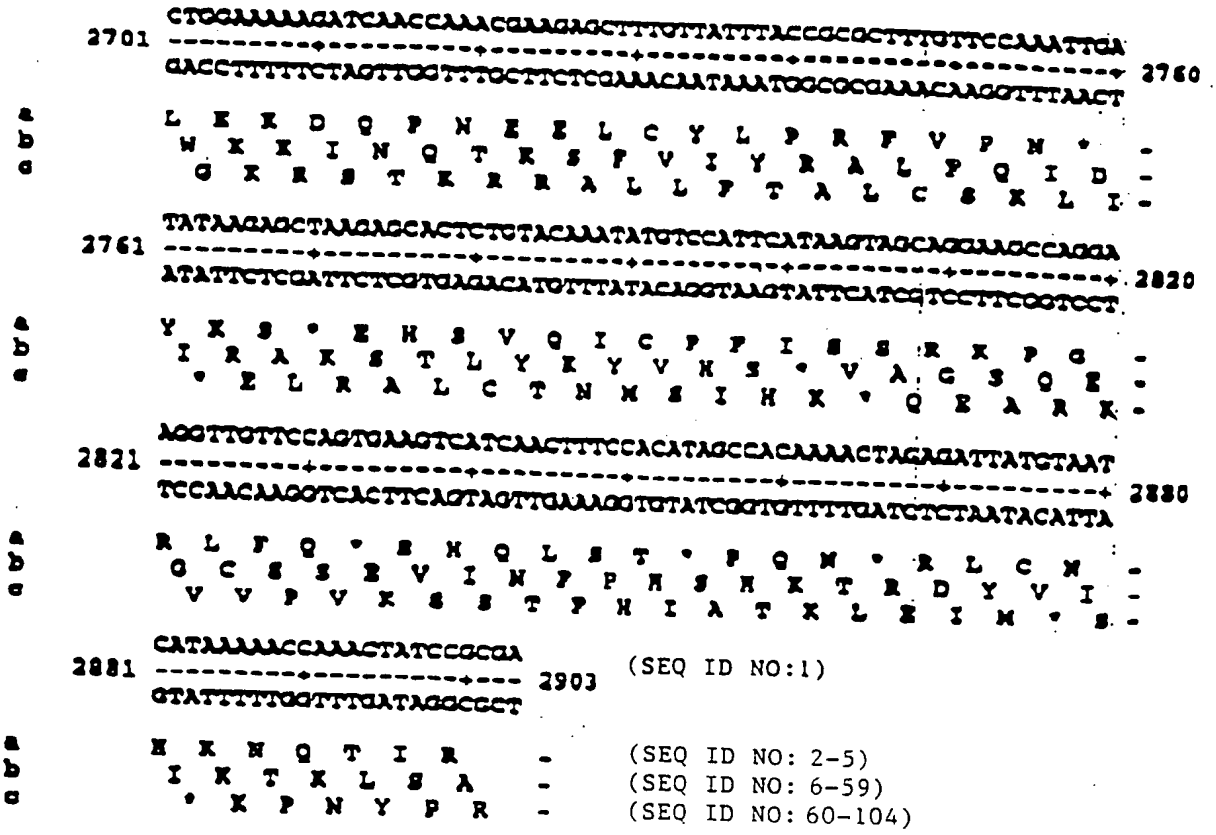

FIG. 2 CONTINUED

```
...TGATCTCTGGCTCAGTGCCAGTAGTCCATTTGAGAGCAA...CGTAGCCCCCGCGTG   -86

GCGCATCATGGAGCTATTTCGGAATTTTCGCAGGGTTATCGATTCGTAGTGGGAACCCATT   -26

                                1
CATTGTTTGGAACCACCAACGGACGACTTAACAAGCTCCCCGAGGTGCATGATGAAAATT    35
                                                    MetLysIle

GCTCCAGTTGCCATAAATCACAGCCCGCTCAGCAGGGAGGTCCCGTCACACGCGGCACCC    95
AlaProValAlaIleAsnHisSerProLeuSerArgGluValProSerHisAlaAlaPro

ACTCAGGCAAAGCAAACCAACCTTCAATCTGAAGCTGGCGATTTAGATGCAAGAAAAAGT   155
ThrGlnAlaLysGlnThrAsnLeuGlnSerGluAlaGlyAspLeuAspAlaArgLysSer

AGCGCTTCAAGCCCGGAAACCCGCGCATTACTCGTACTAAGACAGTACTCGGGAGACAC   215
SerAlaSerSerProGluThrArgAlaLeuLeuAlaThrLysThrValLeuGlyArgHis

AAGATAGAGGTTCCGGCCTTTGGACGGTGGTTCAAAAAGAAATCATCTAAGCACGAGACG   275
LysIleGluValProAlaPheGlyGlyTrpPheLysLysLysSerSerLysHisGluThr

GGCGGTTCAAGTGCCAACGCAGATAGTTCGAGCGTGGCTTCCGATTCCACCGAAAAACCT   335
GlyGlySerSerAlaAsnAlaAspSerSerSerValAlaSerAspSerThrGluLysPro

TTGTTCCGTCTCACGCACGTTCCTTACGTATCCCAAGGTAATGAGCGAATGGGATGTTGG   395
LeuPheArgLeuThrHisValProTyrValSerGlnGlyAsnGluArgMetGlyCysTrp

TATGCCTGCGCAAGAATGGTTGGCCATTCTGTCGAAGCTGGGCCTCGCCTAGGGCTGCCG   455
TyrAlaCysAlaArgMetValGlyHisSerValGluAlaGlyProArgLeuGlyLeuPro

GAGCTCTATGAGGGAAGGGAGGCGCCAGCTGGGCTACAAGATTTTTCAGATGTAGAAAGG   515
GluLeuTyrGluGlyArgGluAlaProAlaGlyLeuGlnAspPheSerAspValGluArg

TTTATTCACAATGAAGGATTAACTCGGGTAGACCTTCCAGACAATGAGAGATTTACACAC   575
PheIleHisAsnGluGlyLeuThrArgValAspLeuProAspAsnGluArgPheThrHis
```

Fig. 3

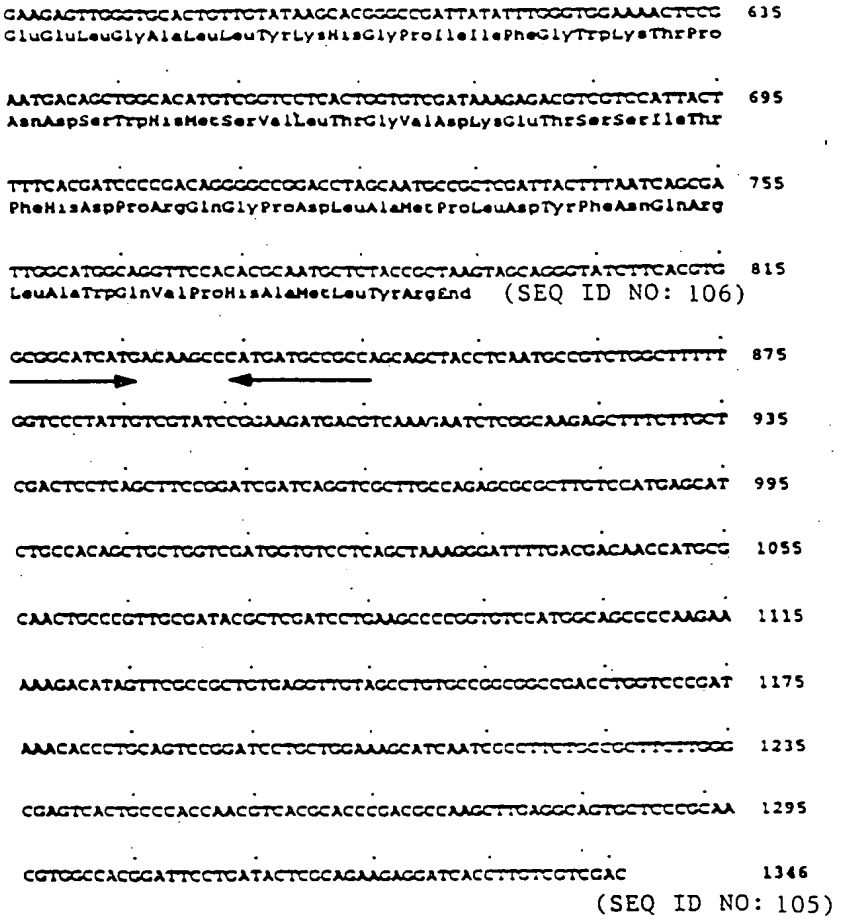

Fig. 3 (continued)

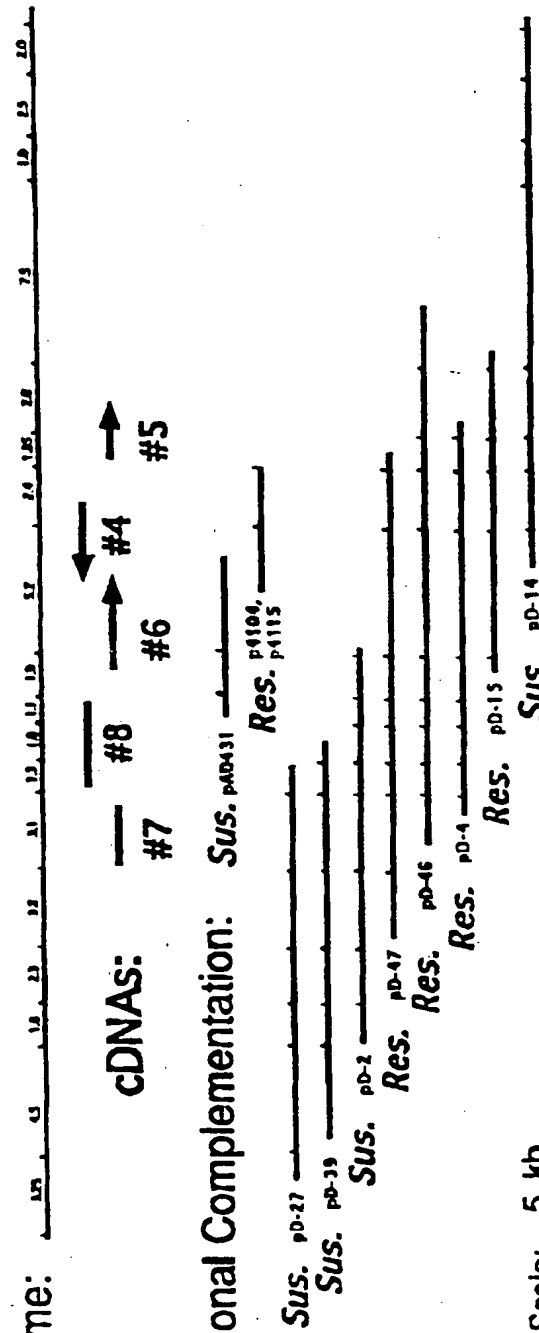
RPS2
Genome:
cDNAs:
#7
#8
#6
#4
#5
Functional Complementation:
Sus. pAD431
Res. p1104, p1115
Sus. pD-27
Sus. pD-39
Sus. pD-2
Res. pD-47
Res. pD-46
Res. pD-4
Res. pD-15
Sus. pD-14
Scale: 5 kb

Figure 4.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04570

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/04, 15/29, 15/31, 15/82; C07K 14/415; A01H 4/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/172.3, 320.1, 240.4, 69.1, 70.1; 530/370; 800/205, DIG 15; 536/23.6, 23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MPSRCH, SDC
search terms: SEQ ID NOS: 1-5 AND 105

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Molecular and Biochemical Parasitology, Volume 59, issued 1993, Dalrymple, et al, "Cloning and characterization of cDNA clones encoding two Babesia bovis proteins with homologous amino- and carboxy-terminal domains", pages 181-189, especially see sequence figures.	7
X	The Plant Cell, Volume 3, issued January 1991, M.C. Whalen et al, "Identification of Pseudomonas syringae pathogens of Arabidopsis and a bacterial locus determining avirulence on both Arabidopsis and soybean", pages 49-58, see especially page 58.	39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 JUNE 1995	Date of mailing of the international search report 13 JUL 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHE SWYDEN CHERESKIN Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04570

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The Plant Cell, Volume 5, issued August 1993, B.N. Kunkel et al, "RPS2, and Arabidopsis disease resistance locus specifying recognition of Pseudomonas syringae strains expressing the avirulence gene avrRpt2", pages 865-875, see the entire document.	1-40
Y	Phil. Trans. R. Soc. Lond. B, Volume 342, Number 1301, issued 29 November 1993, C. Dean, "Advantages of Arabidopsis for cloning plant genes", pages 189-195, see especially Table 1.	1-40
A	Molecular Plant-Microbe Interactions, Volume 3, Number 2, issued 1990, D.Y. Kobayashi et al, "Molecular characterization of avirulence gene D from Pseudomonas syringae pv. tomato", pages 94-102, see the entire document.	1-40
A	Molecular Plant-Microbe Interactions, Volume 3, Number 2, issued 1990, D.Y. Kobayashi et al, "A gene from Pseudomonas syringae pv. gylcinea with homology to avirulence gene D from P.s. pv. tomato but devoid of the avirulence phenotype", pages 103-111, see the entire document.	1-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04570

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/172.3, 320.1, 240.4, 69.1, 70.1; 530/370; 800/205; 536/23.6, 23.7